Bohn, Brent

From: Gibbons, Catherine
Sent: Friday, December 04, 2015 7:36 PM
To: Bohn, Brent
Subject: FW: Cr(VI) bimonthly presentations

From: Gibbons, Catherine
Sent: Friday, October 24, 2014 3:28 PM
To: Khan, Elaine@OEHHA <Elaine.Khan@oehha.ca.gov>
Subject: RE: Cr(VI) bimonthly presentations

Oh I definitely won't, thanks to these! But I'll make up for it next weekend! I love Halloween.

Thanks again for the call about David, I was surprised that the contractors hadn't confirmed with him yet, but apparently they said they'd sent him some emails and never got a response. Not sure if that's true or not.

Thank you!

From: Khan, Elaine@OEHHA [mailto:Elaine.Khan@oehha.ca.gov]
Sent: Friday, October 24, 2014 3:26 PM
To: Gibbons, Catherine
Subject: RE: Cr(VI) bimonthly presentations

Great, thank you! Enjoy your weekend!

From: Gibbons, Catherine [mailto:Gibbons.Catherine@epa.gov]
Sent: Friday, October 24, 2014 12:22 PM
To: Khan, Elaine@OEHHA
Subject: Cr(VI) bimonthly presentations

Greetings! These are all publicly available on the docket, so I thought I'd save you the trouble and send them along. Enjoy!

--Catherine

Bohn, Brent

From: Gibbons, Catherine
Sent: Friday, December 04, 2015 7:32 PM
To: Bohn, Brent
Subject: FW: OEHHA Shop Talk

From: Khan, Elaine@OEHHA [mailto:Elaine.Khan@oehha.ca.gov]
Sent: Monday, January 05, 2015 4:05 PM
To: Gibbons, Catherine <Gibbons.Catherine@epa.gov>
Subject: OEHHA Shop Talk

Live meeting details:
Attendee URL:
Audio: Two way computer audio conferencing: On, but last time we were hosting the meeting in Sacramento we had a lot of trouble with the computer audio, so you may want instead to use the Telephone conferencing:
Audio conferencing provider:

Please remember to mute your computer audio if using the telephone conferencing.

Bohn, Brent

From: Gibbons, Catherine
Sent: Friday, December 04, 2015 7:31 PM
To: Bohn, Brent
Subject: FW: hypochlorhydria (high stomach pH) in the US population

From: Sasso, Alan
Sent: Thursday, January 15, 2015 8:36 AM
To: Elaine.Khan@oehha.ca.gov
Cc: Gibbons, Catherine <Gibbons.Catherine@epa.gov>
Subject: RE: hypochlorhydria (high stomach pH) in the US population

That's OK, it was more of an FYI than a question.

At 3 or 4 of our public meetings, we asked the public and industry to identify susceptible populations, and somehow nobody mentioned this!

-Alan

From: Khan, Elaine@OEHHA [mailto:Elaine.Khan@oehha.ca.gov]
Sent: Wednesday, January 14, 2015 5:06 PM
To: Sasso, Alan
Cc: Gibbons, Catherine
Subject: RE: hypochlorhydria (high stomach pH) in the US population

Hi, Alan.
Thanks for the info. It looks very interesting. Unfortunately, I've never heard of the condition and I'm racking my brain trying to think if I would know a good person to ask about this. Please let me know if you find out more and vice versa.
Thanks!
Elaine

From: Sasso, Alan [mailto:Sasso.Alan@epa.gov]
Sent: Wednesday, January 14, 2015 1:54 PM
To: Khan, Elaine@OEHHA
Cc: Gibbons, Catherine
Subject: hypochlorhydria (high stomach pH) in the US population

Hi Elaine,

I really enjoyed the talk last week, thanks for sending us the info.

I was reading-up on gastric parameters in the human population (particularly as a function of fed/fasted status), and I saw in this Kalantzi paper, 2 out of the 19 subjects just happened to have a condition called "hypochlorhydria". They persistently have a very high stomach pH, and are very susceptible to gastric cancers and lesions/ulcers (due to biological/bacterial issues, infections, etc).

In 28 hypochlorhydric subjects (Feldman paper), the average basal pH was 7.44 in men,7.65 in women.

In 252 men WITHOUT hypochlorhydria (healthy, not taking medication, etc), 5% of them naturally had a basal/resting (fasted) gastric pH of at least 5.09. in women (n= 113), 5% had pH>=6.81. Those are conditions where our models indicate poor reduction.

So, even without hypochlorhydria, 10% of the population may be above pH=5 .

At the end of the Feldman paper, they say that the true incidence of hypochlorhydria in randomly selected adult humans in the US population is unknown (but that paper is from 1991). I'm having trouble obtaining information on what the incidence may be.

Have you ever heard of this condition?

-Alan

Alan F. Sasso, Ph.D.
Office of Research and Development
National Center for Environmental Assessment
U.S. Environmental Protection Agency
(703)-347-0179

Bohn, Brent

From: Gibbons, Catherine
Sent: Friday, December 04, 2015 7:31 PM
To: Bohn, Brent
Subject: FW: copy of SOT poster
Attachments: Sasso_SOT2015.pdf

From: Sasso, Alan
Sent: Monday, March 30, 2015 8:52 AM
To: Wong, Patty@OEHHA <Patty.Wong@oehha.ca.gov>
Cc: Elaine.Khan@oehha.ca.gov; Gibbons, Catherine <Gibbons.Catherine@epa.gov>
Subject: copy of SOT poster

Hi Patty,

It was nice meeting you at SOT. Here is a copy of my poster from SOT.

Sorry I couldn't find anybody else from your office—SOT is very overwhelming. It's become too big. I spend most of my time there walking miles between posters and talks.

The moral of the story of this poster is that you can obtain the same human equivalent dose using a stomach-only model, as you do with a whole-body PBPK. I believe whole-body PBPK is overkill for this type of problem, and site-specific absorption rates into each GI tissue cannot be validated.

It also shows how high pH individuals may be more susceptible. We don't know the incidence of high stomach pH in the population (since it's an invasive test, usually only done when people are having GI problems).

-Alan

Alan F. Sasso, Ph.D.
Office of Research and Development
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U.S. Environmental Protection Agency
(703)-347-0179

In vivo efficiencies of hexavalent chromium reduction in the gastric environments of mice, rats, and humans

Sasso A.F., Schlosser P.M.

U.S. Environmental Protection Agency, National Center for Environmental Assessment

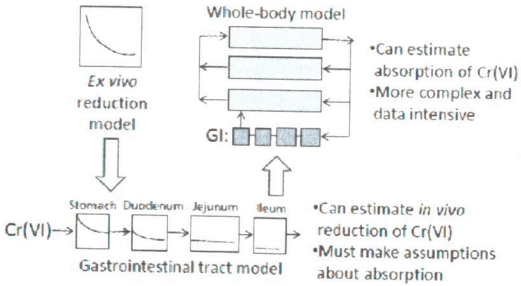
www.epa.gov

Alan Sasso | sasso.alan@epa.gov | 703-347-0179

Introduction

Hexavalent chromium (Cr(VI)) is a known human carcinogen via inhalation, but less is known about human risks via ingestion. Increased incidences of neoplasms in the oral cavity of rats and in the small intestine of mice have been observed in long-term drinking water bioassays (NTP, 2008). When ingested, Cr(VI) can be reduced to trivalent chromium (Cr(III)) within the gastrointestinal (GI) tract. Cr(III) is thought to pose little or no carcinogenic risk. Understanding GI tract reduction is important in evaluating the NTP cancer findings in the context of human health risk assessment.

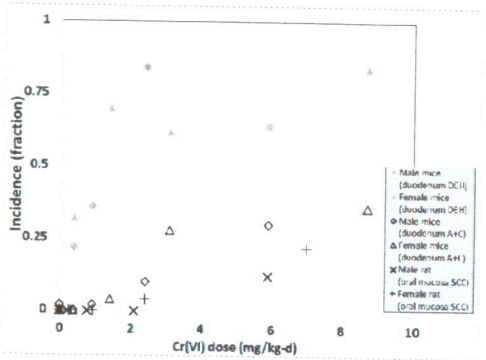
Available toxicokinetic models



Ex vivo models predict reduction under batch conditions. GI tract models incorporate ex vivo models into a dynamic system to estimate in vivo Cr(VI) reduction in the lumen. PBPK models estimate absorption and kinetics of Cr(VI) and Cr(III) in the whole body. The GI tract model in this poster combines the ex vivo model by Schlosser and Sasso (2014) with the stomach compartment of the PBPK model by Kirman et al. (2013).

Toxicity data

NTP (2008) observed diffuse epithelial hyperplasia (DEH) and adenomas and carcinomas (A+C) in the small intestine of mice. The same effects were not observed in rats, although squamous cell carcinomas (SCC) were observed in the oral mucosa.



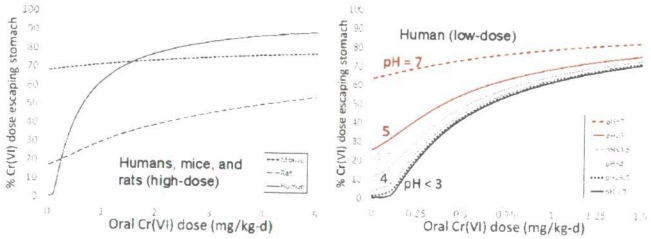
U.S. Environmental Protection Agency
Office of Research and Development

In vivo stomach reduction and GI effects

Reduction of Cr(VI) in the stomach is a major source of inter-species differences. Inefficient reduction results in a greater amount of unreduced Cr(VI) persisting in the small intestinal (SI) compartments (duodenum, jejunum, and ileum).

Species comparison of in vivo stomach reduction

- Toxicokinetic models estimate that rats reduce an equivalent daily Cr(VI) dose more efficiently than mice (on a basis of % unreduced dose escaping stomach).
- At low doses, humans reduce Cr(VI) more efficiently in the stomach than rodents, primarily due to larger stomach size, and lower pH of the human stomach.
- The high efficiency of reduction for humans also leads to a more rapid loss of reducing agent at high doses.



Two potential internal dose metrics for GI tract toxicity are:

1) **absorption** (mg Cr(VI) absorbed per L small intestine tissue), and

2) **pyloric flux** (mg Cr(VI) escaping stomach reduction, per L small intestine tissue). Pyloric flux requires only a GI tract model, while absorption requires a whole body PBPK model.

Despite the relative simplicity of the GI tract model, extrapolating NTP (2008) small intestine toxicity data from mice to humans (using **pyloric flux**) produces similar results as a whole body PBPK model (using **absorption**).

- The averages of the HEDs estimated by a GI tract model range from 0.05–0.1 mg/kg-d, depending on response rate and uncertainty factor (Table 1).
- The human equivalent dose (HED) estimated by a PBPK model was 0.06 mg/kg-d (Thompson et al., 2014).

Table 1. Preliminary dose-response and human extrapolation for diffuse epithelial hyperplasia in mice (using NTP 2008 data)

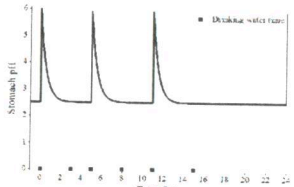
	Pyloric flux (lifetime average mg/L-d)	UF*	Adjusted pyloric flux (mg/L-d)	HED pH=2.5 (mg/kg-d)	HED pH=5 (mg/kg-d)	Average HED (mg/kg-d)
	Mouse simulations			Human simulations		
	Male mouse†					
BMDL5	1.3	3	0.43	0.12	1.4e-2	6.7e-2
BMDL10	2.6	3	0.88	0.14	2.8e-2	8.4e-2
	Female mouse‡					
LOAEL	4.0	30	0.13	8.9e-2	4.5e-3	4.7e-2
		10	0.40	0.12	1.3e-2	6.7e-2
		3	1.3	0.15	4.2e-2	9.6e-2

*Uncertainty factors.
†Male mouse internal dose BMDLs adjusted by UF of 3 to compare with Thompson et al. (2014).
‡Data for the female mouse not amenable to BMD modeling. LOAEL was adjusted by UF of 3, 10, and 30 to account for LOAEL to NOAEL extrapolation and interspecies variation. These UF values were applied for model evaluation purposes only and do not reflect an evaluation of the toxicology data for the mouse.

Benchmark dose (BMD): Dose producing a predetermined change in response rate of effect.
BMDL: Lower confidence limit on the dose at the BMD
LOAEL: Lowest observed adverse effect level

Food effects on Cr(VI) reduction

The pH of human gastric juice spikes to 6 during meals, and returns to baseline within 2 hours (Mudie et al., 2010, Parrott et al., 2009). Simulations were performed that incorporated these pH spikes, as well as changes in gastric emptying rate (KLSD) to assess the impact on average daily internal dose.



Simulation of dietary spikes in stomach pH. It was assumed that the 3 largest drinking water events were associated with a meal.

Table 2. Preliminary human extrapolation for diffuse epithelial hyperplasia in mice

Pyloric flux (mg/L-d)	flux/UF (mg/L-d)	HED (mg/kg-d)			
		pH=2.5	pH=4.9‡ KLSD=1	pH↑↓	pH↑↓ KLSD↓
4.0	0.40*	0.12	2.0e-2	1.8e-2	7.2e-2
	0.13†	8.9e-2	6.9e-3	6.2e-3	2.5e-2

*UF=10; †UF=30 (for evaluation only; see Table 1)
‡Default constant GastroPlus™ model parameters for fed human.
pH↑↓: Dynamic human pH (baseline of 2.5, spikes to 6 with meals).
KLSD↓: Constant low stomach emptying rate (KLSD=0.415/hr).

Model predictions for fed humans have higher uncertainty, and may overestimate the human internal dose because:

- Kinetic model was based on parameters derived from fasted human gastric samples
- Gastric juice of humans in the fed state will have higher reducing capacity
- Gastric emptying rate decreases during solid meals (increasing stomach reduction)

However, both the decreased reduction rate and decreased stomach emptying may increase Cr(VI) exposure to the stomach epithelium.

Discussion

- A simple 1-compartment GI tract model predicts HEDs similar to those obtained with more complex PBPK models for the endpoint of diffuse epithelial hyperplasia in mice.
- Reduction efficiencies predicted by GI tract models are consistent with NTP (2008)
  - Mice are more susceptible than rats to effects in the small intestine.
- Net effect of fed status on Cr(VI) stomach kinetics in humans is unknown.
- Analyses incorporating population variability and uncertainty of human and rodent gastric parameters is the subject of ongoing work.

Acknowledgments

The authors thank Ravi Subramaniam, Catherine Gibbons, Susan Rieth, Amanda Persad, Elaina Kenyon, Chris Brinkerhoff, Weihsueh Chiu, and Vincent Cogliano for helpful comments and discussions.

Disclaimer: The views expressed in this poster are those of the authors and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency, nor does mention of trade names constitute endorsement or recommendation for use.

References

Mudie et al., (2014). Quantification of gastrointestinal liquid volumes and distribution following a 240 ml dose of water in the fasted state. Mol Pharm

NTP (2008). NTP Technical Report on the Toxicology and Carcinogenesis Studies of Sodium Dichromate Dihydrate in F344/N Rats and B6C3F1 Mice (Drinking Water Studies).

Parrott et al., (2009). Predicting pharmacokinetics of drugs using physiologically based modeling--application to food effects. AAPS J 11: 45-53.

Kirman et al., (2013). Physiologically based pharmacokinetic model for humans orally exposed to chromium. Chem Biol Interact 204: 13-27.

Schlosser, PM; Sasso, AF. (2014). A revised model of ex-vivo reduction of hexavalent chromium in human and rodent gastric juices. Toxicol Appl Pharmacol 280: 352-361.

Thompson et al., (2014). A chronic oral reference dose for hexavalent chromium-induced intestinal cancer. J Appl Toxicol 34: 525-536.

Bohn, Brent

From: Gibbons, Catherine
Sent: Friday, December 04, 2015 7:31 PM
To: Bohn, Brent
Subject: FW: stomach parameters for mice
Attachments: McConnell et al. 2008 GI tract pH.pdf

From: Sasso, Alan
Sent: Wednesday, April 22, 2015 2:42 PM
To: Elaine.Khan@oehha.ca.gov
Cc: Gibbons, Catherine <Gibbons.Catherine@epa.gov>
Subject: stomach parameters for mice

Hi Elaine,

I just wanted to follow-up on something I mentioned yesterday. Attached is a paper on the stomach parameters in rats and mice.

These data are used by GastroPlus to generate stomach/GI pH values for pharmaceutical development. The stomach pH for mice is 2.98 for the fed state, and 4.04 for the fasted state.

The Kirman/Proctor papers assumed a stomach pH of 4.5 for mice. Looking at their papers, they say that when they collected gastric juice from rodents, the pH was initially about 4.0, and rose to 4.5 with the addition of DI water. But they keep the value at pH 4.5 for their whole risk assessment and never change the assumption.

Mice in the "fed" state from the McConnell paper were simply administered food and drinking water ad libitum before sacrifice, so the fed state might best represent the stomach pH during the NTP study. A few other papers note that drinking water usually occurs at times of meal events.

If this is true, then more reduction occurs in the mouse stomach than previously thought. As a result, they are experiencing effects at lower internal doses than previously thought. This has the effect of lowering the human equivalent dose following animal-human extrapolation.

But another caveat- this makes the mouse stomach/GI TK data look more nonlinear than before.

This job is hard....

-Alan

JPP Journal of Pharmacy and Pharmacology

JPP 2008, 60: 63–70

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DOI 10.1211/jpp.60.1.0008
ISSN 0022-3573

Measurements of rat and mouse gastrointestinal pH, fluid and lymphoid tissue, and implications for in-vivo experiments

Emma L. McConnell, Abdul W. Basit and Sudaxshina Murdan

Abstract

To use rodent models effectively in in-vivo investigations on oral drug and vaccine delivery, the conditions in the gastrointestinal tract must be understood. Some fundamental information is currently unavailable or incomplete. We have investigated the pH, water content and lymphoid tissue distribution along the gastrointestinal tract, as well as the stomach volume, as these were critical to our investigations on pH-responsive drug delivery and colonic vaccination. The observed values were compared with those in man as an indication of the validity of the rodent model. The mouse stomach pH was 3.0 (fed) and 4.0 (fasted), and the corresponding values in the rat were 3.2 (fed) and 3.9 (fasted). The mean intestinal pH was lower than that in man (< pH 5.2 in the mouse; < pH 6.6 in the rat). This brings into question the use of rodents in investigations on enteric-coated drug carriers targeted to the large intestine/distal gut. The water content in the gastrointestinal tract in the fed and fasted mouse was 0.98 ± 0.4 and 0.81 ± 1.3 mL, respectively, and in the fed and fasted rat was 7.8 ± 1.5 and 3.2 ± 1.8 mL. When normalized for body weight, there was more water per kg body weight in the gastrointestinal tracts of the mouse and rat, than in man. The stomach capacity was found to be approximately 0.4 and 3.4 mL for mice and rats, respectively. The low fluid volume and stomach capacity have implications for the testing of solid dosage forms in these animal models. Substantial amounts of lymphoid tissue analagous to small intestinal Peyer's patches were measured in the rat and mouse colon, showing the feasibility of colonic vaccination, a route which might prove to have different applications to the more commonly studied oral vaccines. The existence of lymphoid tissue in the mouse and rat caecum has also been reported.

Introduction

Animal models are used extensively in the pre-clinical testing of drugs and vaccines. Rodents (mainly rats and mice) are often used due to their small size and low cost. Rats, having a relatively larger size and greater capacity for blood samples, are more useful for bioavailability studies, whereas mice are often used for vaccination studies. Despite the extensive use of these animals, certain features are either unknown or inadequately characterized, although a number of aspects of the mouse and rat gastrointestinal (GI) physiology have been reviewed by Kararli (1995). During our investigations into pH-responsive drug release at different locations in the gastrointestinal tract, and into colonic vaccination, we identified several key elements of gastrointestinal physiology that needed clarification to enable the use of rat and mouse models in the in-vivo studies. These were the pH and fluid content along the gastrointestinal tract, the stomach volume and the presence of lymphoid tissue in the colon of the animal models.

The pH in the gastrointestinal tract is a crucial factor, affecting the stability and solubility of drugs and their absorption through the mucosa; unsuitable pH may cause the precipitation of acidic or basic drugs from solution, or the degradation of labile compounds. In addition, enteric-coated drug delivery systems for modified or targeted drug release are increasingly being investigated, for example using polymers such as polymethacrylate- and cellulose-based enteric coatings, which dissolve only when the pH of the environment exceeds a threshold level. In such a situation, knowledge of the

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Emma L. McConnell, Abdul W. Basit, Sudaxshina Murdan

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gut pH of the experimental animal is critical. Previous reports on the pH of the rat gastrointestinal tract are conflicting (Smith 1965; Ward & Coaes 1987), while reports of pH in the mouse small and large intestinal tract were not found.

The fluid content of the gastrointestinal tract is another critical factor in the dissolution of drug from a dosage form, and the dispersion of solid-dosage forms. Lomas & Graves (1999) and Schiller et al (2005) suggested that water in the gut lumen of man was not homogeneously distributed; this implied that a dosage form would be in contact with varying amounts of fluid or indeed none at all during its passage through the gastrointestinal tract. To enable better animal study design and extrapolation to man, or to better explain dosage form/drug behaviour in the rodent model, knowledge of the water content in these animals is important.

In this study, we have investigated the stomach volume, fairly crudely, to give a rough indication of the volumes that may be administered orally to the animal models. To our knowledge, there are no reports of mouse and rat stomach volume, although maximum volumes to be administered by the oral route have been suggested (Wolfensohn & Lloyd 1994). Gelatin capsule shells and mini-tablets have been administered to rats (Hu et al 1999; Wong et al 2006). Knowledge of the animal stomach volume would enable calculation of dosage form:stomach volume ratio, which would give an indication of the likely fate of the dosage forms, with respect to disintegration and drug dissolution and absorption.

In our laboratories, we are also investigating colonic vaccination as it may have different applications to the more commonly studied oral vaccines, which are expected to be processed mainly by the small intestinal immunological system. Like the small intestine, the colon contains gut-associated lymphoid tissue. In man, there are approximately 339 Peyer's patches (Cornes 1965) in the small intestine, and approximately 12 000–18 000 follicles in the large intestine (Langman & Rowland 1986, 1992; Gebbers et al 1992). Presence of such a large number of follicles in man's colon implies the feasibility of vaccine uptake and processing in the colon. Very little is known, however, about colonic vaccine uptake, although significant differences between the large and small intestinal immunological environments have been reported. For example, a predominance of IgA2 cells over IgA1 cells is seen in the colon (as in the rectum and in the female genital tract) in contrast to a predominance of IgA1 cells in the small intestine (McGhee et al 1999). Other benefits of colonic targeting include the decreased proteolytic activity which may be beneficial for sensitive antigens and the higher transit time, which could lead to prolonged antigen contact with the lymphoid tissue and thereby increased uptake. Before mice and rats can be used in studies on colonic vaccination, the presence and density of lymphoid tissue in the colon must be established. Although the lymphoid tissue in the small intestine of mice and rats has been well quantified (Hillery et al 1994; Florence et al 1995; Abe & Ito 1977), the lymphoid tissue in the large intestine has not, and has been reported here.

Materials and Methods

Animals

All procedures were approved by the School's Ethical Review Committee and were conducted in accordance with the Home Office standards under the Animals (Scientific Procedures) Act 1986.

Adult female Balb/c mice (18–22 g) and adult female Wistar rats (160–190 g) were purchased from Harlan Olac Ltd. The animals were fed on Teklad Global 18% Protein Rodent Diet, from Harlan Olac Ltd.

Preparation and dissection procedure

Groups of animals (n = 5–8) were fasted overnight with free access to water, while other groups were allowed access to food and water at all times. The mice were killed by a Schedule One method (CO_2 asphyxiation), after which the intestinal tract was immediately removed and divided into sections: the stomach, the small intestine (into three sections approximating to the duodenum, jejunum and ileum), the caecum and the colon (into two sections approximating to the proximal and distal colon). Subsequently, the pH, water content and lymphoid tissue density of the different sections was measured as follows.

Determination of pH of gastrointestinal contents

The contents of each gastrointestinal section were removed, mixed and the pH was determined using a pre-calibrated pH 211 Microprocessor pH Meter (Hanna Instruments). pH measurements were taken a total of three times with the gastrointestinal tract contents being re-mixed, the pH meter being washed with distilled water and the calibration checked between measurements. An HI 1333 probe was used, with a spherical tip (diameter 7.5 mm); it was ensured that the sample covered the probe tip, and a stable reading acquired. The order in which the pH of the different gastrointestinal tract sections was read was varied within each group to minimize any influence of post-mortem time on pH.

Determination of pH of standard rat/mouse chow

To determine the influence of the animal feed on the pH of the gastrointestinal contents, the pH of standard rat/mouse chow was measured. Three pieces of standard mouse/rat chow (9.17 g) were mixed with 10 mL of tap water until the food pellet had disintegrated, and the pH of the resulting mixture was measured using the same pH meter.

Determination of water and solid contents of the gastrointestinal tract

To determine the gastrointestinal water and solid contents, the wet mass of the section contents was recorded, followed by lyophilization (Virtis-Advantage Freeze Drying Apparatus, Virtis, UK), measurement of the dry mass and calculation of water content.

Determination of stomach capacity

Approximate values for the volume of the mouse and rat stomach were determined by filling the stomach with distilled

water, and observing the results. The aim was to produce a rough estimate, as the method could only give a crude assessment of the volume, and was subject to investigator bias. The stomach, hand-held shut at the pyloric opening, was filled, using a syringe, via the oesophagus until it was considered to be comfortably full, with no obvious stress on the tissue (1), stretched (2), or to the point of bursting (or could no longer be filled) (3).

Determination of lymphoid tissue patches along the gastrointestinal tract

The method of Langman & Rowland (1986) was used. The emptied gastrointestinal sections were placed into glass vials containing 20 mL 10% v/v aqueous acetic acid and incubated overnight in the refrigerator. Acetic acid was used as it enhanced the visualization of the lymphoid tissue. The following day, the gastrointestinal tract sections were removed, opened lengthways, blotted dry and photographed, and the numbers of individual lymphoid follicles and patches (collections of follicles) were counted. The mean number of patches or follicles per cm was calculated from the data for the individual animals.

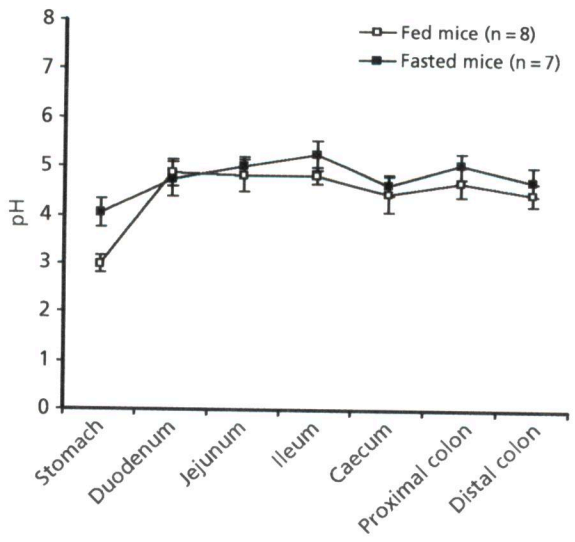

Figure 1 pH values along the mouse gastrointestinal tract. Mean and error bars are shown.

Statistical analysis

The data gathered from mice was analysed using parametric tests. The influence of fed (n = 8) and fasted (n = 7) states on mouse gastrointestinal pH, and water and solid contents were analysed using Student's Independent *t*-test. Differences between gastrointestinal tract sections for pH and water content were analysed using one-way analysis of variance, with post-hoc analysis using Tukey's test.

The data obtained from rats was analysed using non-parametric tests, as the data did not fulfil the assumptions required for parametric tests. The influence of fed (n = 5) and fasted (n = 5) state on rat gastrointestinal pH, and water and solid contents were analysed using the Mann–Whitney *U*-test. The differences between gastrointestinal sections for pH and water content were analysed using Kruskal–Wallis, with Nemenyi's post-hoc analysis.

All tests, apart from Nemenyi's test were carried out using SPSS Version 14.0 statistical software package. Nemenyi's test was conducted as described in Jones (2002). Results were considered statistically significant when $P < 0.05$.

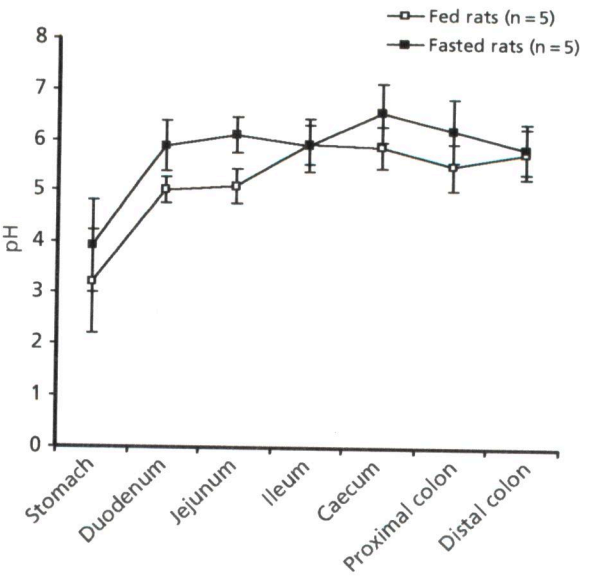

Figure 2 pH values along the rat gastrointestinal tract. Mean and error bars are shown.

Results and Discussion

The pH along the gastrointestinal tract of mice and rats

The pH of the contents of the different gastrointestinal sections of fed and fasted mice and rats are shown in Figures 1 and 2, and in Table 1. The standard deviations showed variability between individuals. Such variability has been observed in man (Evans et al 1988; Fallingborg et al 1989). The lowest pH was seen in the stomach, in both rats and mice. In both animals, the stomach pH appeared higher in the fasted state (3.9 compared with 3.2 in rats and 4.0 compared with 3.0 in mice), although the difference was only statistically significant in the mouse. Higher pH in the fasted state was surprising given that, in man, the fasted gastric pH is lower than the fed gastric pH (fasted pH 1.7 increasing to 5.0 after meal ingestion in healthy subjects (Dressman et al 1990; Russell et al 1993)) due to the buffering effects of food (Malagelada et al 1976). However, this was dependent on the meal type, with high protein meals having increased buffering effect over an isocalorific carbohydrate meal (Richardson et al 1976). In this study, the mice and rats were fed on a standard low protein (18%), low fat (5%) diet. The low

Table 1 The pH values of the mouse and rat gastrointestinal tract

Gastrointestinal section	pH mean (s.d.)			
	Mice		Rats	
	Fed	Fasted	Fed	Fasted
Stomach	2.98 (0.3)	4.04 (0.2)	3.20 (1.0)	3.90 (1.0)
Duodenum	4.87 (0.3)	4.74 (0.3)	5.00 (0.3)	5.89 (0.3)
Jejunum	4.82 (0.2)	5.01 (0.3)	5.10 (0.3)	6.13 (0.3)
Ileum	4.81 (0.3)	5.24 (0.2)	5.94 (0.4)	5.93 (0.4)
Caecum	4.44 (0.2)	4.63 (0.4)	5.90 (0.4)	6.58 (0.4)
Proximal colon	4.69 (0.3)	5.02 (0.3)	5.51 (0.5)	6.23 (0.4)
Distal colon	4.44 (0.3)	4.72 (0.2)	5.77 (0.5)	5.88 (0.5)

protein content of the animals' diet could be responsible for the absence of a food buffering effect. The pH of rat chow in water was 5.86 ± 0.06 and was therefore not responsible for the lower pH observed in the fed state. In addition, while the reasons for the difference between man and rodents are not clear, it is obvious that during experiments in man, fed and fasted states can be controlled more closely. In contrast, although the fed-state mice have free access to food, it is not known at what time they last ingested food, and in what quantity, and the immediate buffering effects of food may not have been observed.

The pH of the small intestinal contents also appeared to be higher in the fasted state than in the fed state, but this was not statistically significant in rats or mice. This suggested that the fed state of the animal had no effect on intestinal pH, which is similar to the situation in man, where the small intestinal and colonic pH are variable, but differences are not largely associated with the fed or fasted states (Kalantzi et al 2006). As expected, the small intestinal pH was higher than the gastric pH, due to the secretion of pancreatic juice and buffering with bicarbonate ions. In mice, there was a small drop in pH in the caecum. This may be associated with the increased presence of short chain fatty acids produced by bacterial polysaccharidases, bacteria being present in greater numbers in the caecum. Such a pH drop in the caecum also occurs in man (Evans et al 1988). Overall, the mean intestinal pH of both mice and rats does not reach the pH values reported in man i.e. 7.5, 6.4 and 7 in the distal small intestine, caecum and colon, respectively (Evans et al 1988).

The stomach pH values for the rat and mice were similar to that reported by Smith (1965). In contrast, the mean intestinal pH values of both animals were lower than expected and did not reach the values of pH 6–8 that have been reported in the literature for rats (Smith 1965; Ward & Coates 1987), though some individual rat pH values were found to be above pH 7. Differences in the methodology may help explain the different values obtained. Smith (1965) mixed distilled water with rat gut contents, while Ward & Coates (1987) inserted a pH probe into sections of excised rat gastrointestinal tract. In our investigation, mixing of undiluted contents was carried out, which may be more representative of the pH that a drug or delivery system is exposed to, due to the continually moving intestinal contents. To our knowledge, this is the first report of the pH of mouse intestinal tract contents.

The low intestinal pH in mouse and rat has implications for the in-vivo testing of oral pharmaceuticals in these animals. For example, drugs which require a basic pH to dissolve may precipitate at the lower pH values seen in the mouse or rat. This may prevent drug absorption and pharmacokinetic extrapolation to man would be inaccurate. The lower pH seen in mice and rat gastrointestinal tract also has implications when pH-responsive drug carriers are being investigated. For example, the pH responsive polymethacrylate polymers such as Eudragit S and FS, which dissolve at pH 7.0, but are water-insoluble at lower pH, are being investigated to target drug release to the distal intestinal tract e.g. for the treatment of diseases such as ulcerative colitis (Basit 2005; Ibekwe et al 2006). The low pH values for the mouse and rat gastrointestinal tract shown in this paper ($pH < 7.0$) suggest that rats and mice may not be the most appropriate models for the study of pH sensitive dosage forms targeted to the human lower intestine and colon, where pH is often greater than 7.0.

The water and solid contents of the mouse and rat gastrointestinal tract

The contents of the gastrointestinal tract are generally semi-solid. Water, either ingested or secreted, exists as fluid in the gastrointestinal tract. In this study, we measured the water content by freeze drying; the solid and water contents of the gastrointestinal tract of mice and rats are shown in Figures 3 and 4, respectively. As expected, total contents of the gastrointestinal tract were greater in rats than in the smaller mice. There were more solid contents in the fed rat gastrointestinal tract than in the fasted rat. Water content was also higher in the fed state, possibly due to increased secretions and water bound with the ingested food. The total amount of water present in the rat gastrointestinal contents was similar to that reported by Cizek et al (1954), who measured water by evaporating gastrointestinal contents to dryness and reported that gut water represented 1.8% (fasted) and 4.5% (fed) of total body weight (198–232 g) of female rats. In the mouse, differences between the total solid and water contents were less

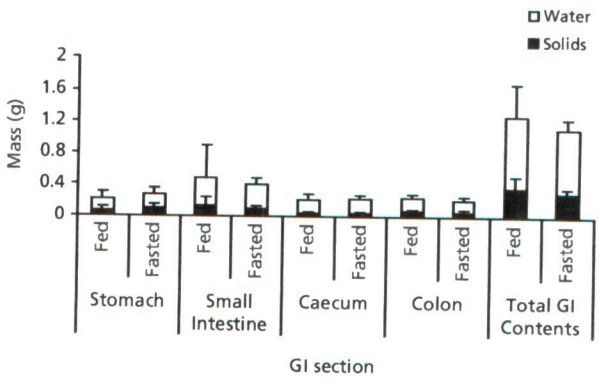

Figure 3 Water and solid compositions of the mouse gastrointestinal tract contents. Mean and error bars are shown.

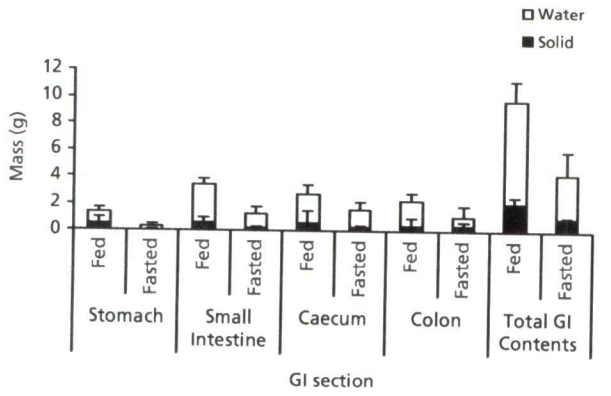

Figure 4 Water and solid content of the rat gastrointestinal tract contents. Mean and errors bars are shown.

obvious between the fed and fasted states, the small quantities making it more difficult to ascertain differences. As expected, the water concentration decreased along the length of the gastrointestinal tract from 82% w/w of the small intestinal contents to 71% w/w in the colon in rats, and from 74 to 67% w/w along the same segments in mice. The decreasing water content was observed visually as an increase in the viscosity of the gastrointestinal contents.

In Figures 3 and 4, the most striking observation was the very low levels of fluid present along the gastrointestinal tract, the total mass of water in the mouse gut being less than 1 mL (0.98 ± 0.4 mL fed, 0.81 ± 1.3 mL fasted). In experiments where mice are orally dosed with solid or semi-solid drug delivery systems, the latter may not come into contact with enough fluid to disperse and/or dissolve. The rat seems a more appropriate model for the dissolution of drug delivery systems, which require contact with sufficient water. The larger water content in the fed rat (7.8 ± 1.5 mL), compared with the fasted rat (3.2 ± 1.8 mL) suggests that if a dosage form is being investigated in the rat model, it may be beneficial to deliver it in the fed state, although interactions of food with drug or with dosage form may mean that this is not appropriate in all circumstances.

To compare with human data, the mass of rodent intestinal contents with respect to total body mass has been calculated. In man, the total large intestinal (colonic and caecal) water content post-mortem was found to average 187 g, or 2.6 g kg^{-1} body mass assuming a 70-kg body weight. For an average rat (175 g), the average (fed and fasted) colonic water content was 7.14 g kg^{-1} or 16.9 g kg^{-1} when the caecum was included. For an average (fed and fasted) mouse, the values were 7.8 g water kg^{-1} body weight and 16.3 g kg^{-1} when the caecal contents were counted. In man, the small intestine has been reported to contain a total of 206 g water or 3.8 g kg^{-1} (Gotch et al 1957). This compared with 11.1 g water kg^{-1} body weight in the rat small intestine, and 16.5 g water kg^{-1} body weight in the mouse small intestine. The same authors found 118 g water in the stomach or 2.2 g water kg^{-1} body weight. In our study, the corresponding values were 3.2 g kg^{-1} in rats and 8.5 g water kg^{-1} body weight in mice. Thus, when the values were normalized to take into account total body mass, more water per kg body weight was found in the gastrointestinal tracts of the mouse and the rat than in man.

Interestingly, although the total water content reported in the small and large intestine in man was high (206 g (Gotch 1957) and 187 g, respectively (Cummings et al 1990)), Schiller et al (2005), using magnetic reasonance imaging, measured a median free fluid volume of 105 ± 72 mL (fasted) and 54 ± 41 mL (fed) in the small intestine, and 13 ± 12 mL (fasted) and 11 ± 26 mL (fed) in the colon. These values indicated that most of the gut water was in the bound state. This suggested that only a proportion of the water content was available for drug or dosage form dissolution, and the same is likely to be true of the water content in the animal models discussed.

The volume of the mouse and rat stomach

Drug or vaccine formulations are often given to experimental animals by oral gavage. Consequently, the volume of the stomach is considered an important parameter for oral dosing, and the results are shown in Table 2. The mouse stomach was approximately one-tenth the volume of the rat stomach. Wolfensohn & Lloyd (1994) have suggested the upper limit for oral dosing in mice to be 20 mL kg^{-1}. Thus, for a mouse of 20 g, the maximum oral dosage volume would be 0.4 mL. For rats, the recommended maximum is 10 mL kg^{-1}; for a 200 g rat this would give a dosing volume of 2 mL. These values correlate to some degree with the 'comfortably full' volumes shown in Table 2, despite the fact that post-mortem results would be likely to differ from an in-vivo situation, since elasticity and responsiveness of gastric tissue to pressure may be altered.

Quantification of lymphoid tissue along the gastrointestinal tract of the mouse and rat

The lymphoid tissue along the gastrointestinal tract can be categorized broadly into, firstly, individual lymphoid follicles, which are seen as raised white areas, and secondly into patches, which are collections of individual follicles. In the small intestine these are referred to as Peyer's patches. No lymphoid tissue was observed in the stomach. However, significant amounts of lymphoid tissue were observed in the mouse and rat caecum (Table 3 ; Figure 5A, B). Thus, we confirmed previous reports on the presence of lymphoid tissue in mouse caecum (Owen et al 1991) and have reported, for the first time to our knowledge, the presence of lymphoid tissue in rat caecum.

Table 2 Fill volumes of mouse and rat stomach

	Volume (mL (s.d.))	
	Mice (n = 10)	Rats (n = 8)
1. Comfortably full	0.37 (0.09)	3.38 (0.52)
2. Stretched	0.55 (0.09)	4.63 (0.44)
3. On the point of bursting/could not be expanded further	0.71 (0.11)	6.63 (0.92)

Table 3 Quantification of lymphoid tissue in the intestinal tract of Balb/c mice and Wistar rats. The mean and (range) values are shown

	Mouse (n = 15)			**Rat (n = 10)**		
	Small intestine	Caecum	Colon	Small Intestine	Caecum	Colon
Mean length (range)	34.5 (29–39)	–	11.5 (9–14)	82.8 (70–97)	–	13.9 (12–18)
Mean number of patches (range)	10.1 (3–15)	1.4 (1–5)	11.6 (7–15)	9.4 (7–15)	1.2 (1–2)	3.8 (2–11)
Mean number patches cm^{-1}	0.3	–	0.8	0.33	–	0.3
Mean number of follicles (range)	57.5 (22–80)	18.1 (9–26)	39.4 (18–54)	207.5 (142–273)	13.5 (8–26)	38.6 (16–83)
Mean number follicles per patch	5.7	12.9	3.4	30.6	12	28.5
Mean number follicles cm^{-1}	1.6	–	3.4	2.1	–	3.4

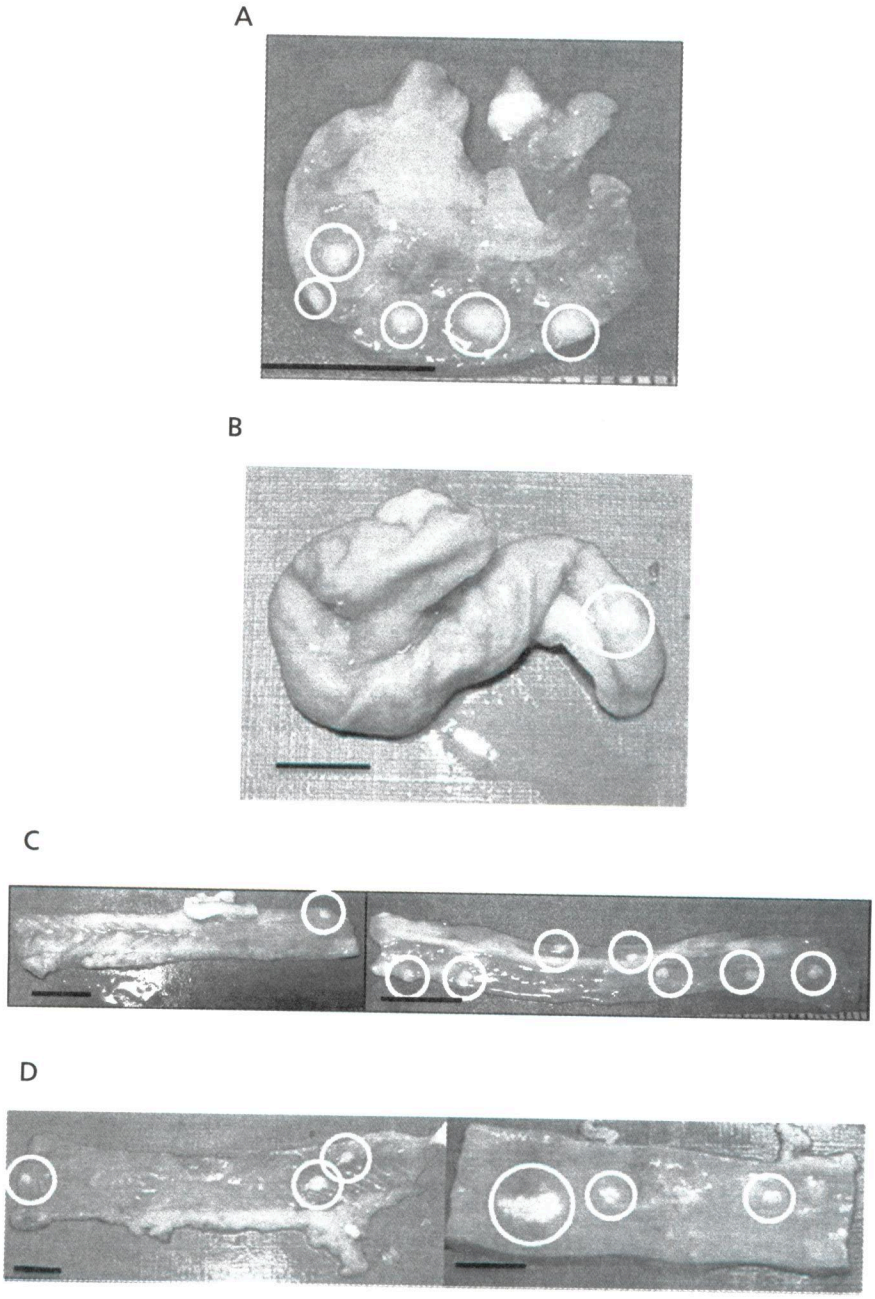

Figure 5 Lymphoid tissue patches in the (A) caecum of a Balb/c mouse (scale bar = 10 mm), (B) caecum of a Wistar rat (scale bar = 10 mm), (C) colon of the Balb/c mouse (left proximal; right distal) (scale bar = 10 mm), (D) colon of the Wistar rat (left proximal; right distal) (scale bar = 10 mm).

The numbers of Peyer's patches in the mouse and rat small intestine (Table 3) were similar to those reported in the literature: 6–12 Peyer's patches in mouse small intestine (Abe & Ito 1977) and 15 Peyer's patches in the rat small intestine (Hillery et al 1994; Florence et al 1995). The values for the number of lymphoid patches in the mouse colon, however, are slightly less than a previously reported value of 1.4 patches cm^{-1} (Owen et al 1991).

Examination of lymphoid tissue density along the gastrointestinal tract revealed that in rats and mice, Peyer's patches were distributed randomly along the sections of the small intestine, with no predilection for a particular area ($P > 0.05$). Examination of the three small intestinal sections (roughly duodenum, jejunum and ileum) within each animal showed that there were similar numbers of patches and follicles per cm, in all three sections (data not shown). Similarly, there was no difference between the number of patches per cm in the proximal and distal colon ($P > 0.05$), which correlated with the random distribution reported in man (Langman & Rowland 1986). Photographs illustrating the random distribution of patches in the mouse and rat colon are shown in Figure 5C, D.

There were, however, differences between the quantity of lymphoid tissue in the small intestine and colon. In mice, the number of patches in the small intestine and colon were similar, but the number of individual follicles was much greater in the small intestine. However, taking into account the lengths of the respective sections, there were actually more follicles and patches per cm in the colon ($P < 0.05$). In rats, there were significantly more patches and follicles in the small intestine, relative to the colon. Taking into account the large differences in intestinal tract length in the rat, similar numbers of patches per cm were seen between small intestine and colon ($P < 0.05$), and more follicles were found per cm in the colon ($P < 0.05$). Mouse colonic lymphoid patches tended to be smaller, containing fewer follicles than small intestinal ones. In contrast, rat lymphoid patches were of similar size in both the small and large intestine. The rat lymphoid patches were, in general, larger than mouse patches and contained a greater number of follicles. The presence of lymphoid tissue in the colon of mice and rats confirms that these animals could be used in colonic vaccination studies.

Conclusion

pH values of the small and large intestinal contents in mice and rats were lower than previously reported, and were lower than the pH levels in man. This has implications for the use of rats and mice in testing of drug formulations, such as pH-responsive drug carriers. The very low levels of fluid present in the mouse gastrointestinal tract cautions against the use of mice when drug dissolution from an oral dosage form is examined. The higher water levels in the rat, especially in the fed state, shows that the rat would be a more suitable animal model. Colonic lymphoid tissue was quantified and compared with small intestinal tissue, in both rats and mice. The significant quantity of lymphoid tissue in the colon in both animals highlights the colon as an immunologically important organ and shows that colonic vaccination may be studied in these animal models. Finally, the presence of lymphoid tissue in the mouse caecum was confirmed and its presence in rat caecum has been reported.

References

Abe, K., Ito, T. (1977) A qualitative and quantitative morphological study of Peyer's patches of the mouse. *Arch. Histol. Japan.* **40**: 407–420

Basit, A. W. (2005) Advances in colonic drug delivery. *Drugs* **65**: 1991–2007

Cizek, L. J. (1954) Total water content of laboratory animals with special reference to volume of fluid within the lumen of the gastrointestinal tract. *Am. J. Physiol.* **179**: 104–110

Cornes, J. S. (1965) Number, size and distribution of Peyer's patches in the human small intestine. *Gut* **6**: 225–229

Cummings, J. H., Banwell, J. G., Segal, I., Coleman, N., Englyst, H. N., Macfarlance, G. T. (1990) The amount and composition of large bowel contents in man. *Gastroenterolgy* **98**: A408

Dressman, J. B., Berardi, R. R., Dermentzoglou, L. C., Russell, T. L., Schmaltz, S. P., Barnett, J. K., Jarvenpaa, K. M. (1990) Upper gastrointestinal (GI) pH in young, healthy men and women. *Pharm. Res.* **7**: 756–761

Evans, D. F., Pye, G., Bramley, R., Clark, A. G, Dyson, T. J., Hardcastle, J. D. (1988) Measurements of gastrointestinal pH profiles in normal ambulant human subjects. *Gut* **29**: 1035–1041

Fallingborg, J., Christensen, L. A., Ingenman-Nielsen, M., Jacobson, B. A., Abildgaard, K., Rasmussen, H. H. (1989) pH-Profile and regional transit times of the normal gut measured by a radiotelemetry device. *Aliment. Pharmcol. Ther.* **3**: 605–613

Florence, A. T., Hillery, A. M., Hussain, N., Jani, P. U. (1995) Nanoparticles as carriers for oral peptide absorption: studies on particle uptake and fate. *J. Control. Release* **36**: 39–46

Gebbers, J. O., Kennel, I., Laissue, J. A. (1992) Lymphoid follicles of the human large bowel mucosa: structures and function. *Verh. Dtsch. Ges. Pathol.* **76**: 126–130

Gotch, F., Nadell, J., Edelman, I. S. (1957) Gastrointestinal water and electrolytes. IV The equilibration of deuterium oxide (D_2O) in gastrointestinal contents and the proportion of total body water (T.B.W) in the gastrointestinal tract. *J. Clin. Invest.* **36**: 289–296

Hillery, A. M., Jani, P. U., Florence, A. T. (1994) Comparative, quantitative study of lymphoid and non-lymphoid uptake of 60 nm polystyrene particles. *J. Drug Target.* **2**: 151–156

Hu, Z., Shimokawa, T., Ohno, T., Kumura, G., Mawatari, S. S., Kamitsuna, M, Yoshikawa, Y., Masuda, S., Takada, K. (1999) Characterization of norfloxacine release from tablet coated with a new pH sensitive polymer, P-4135F. *J. Drug Target.* **7**: 223–232

Ibekwe, V. C., Liu, F., Fadda, H. M., Khela, M. K., Evans, D. F., Parsons, G. E., Basit, A. W. (2006) An investigation into the in vivo performance variability of pH responsive polymers for ileocolonic drug delivery using gamma scintigraphy in humans. *J. Pharm. Sci.* **95**: 2760–2766

Jones, D. (2002) *Pharmaceutical statistics*. Pharmaceutical Press, London

Kalantzi, L., Goumas, K., Kalioras, V., Abrahamsson, B., Dressman, J. B., Reppas, C. (2006) Characterization of the human upper gastrointestinal contents under conditions simulating bioavailability/bioequivalence studies. *Pharm. Res.* **23**: 165–176

Kararli, T. T. (1995) Comparison of the gastrointestinal anatomy, physiology, and biochemistry of humans and commonly used laboratory animals. *Biopharm. Drug Dispos.* **16**: 351–380

Langman, J. M, Rowland, R. (1986) The number and distribution of lymphoid follicles in the human large intestine. *J. Anat.* **194**: 189–194

Langman, J. M., Rowland, R. (1992) Density of lymphoid follicles in the rectum and at the anorectal junction. *J. Clin. Gastroenterol.* **14**: 81–84

Lomas, D. J., Graves, M. J. (1999) Small bowel MRI using water as a contrast medium. *Br. J. Radiol.* **72**: 994–997

Malagelada, J. R., Longstreth, G. F., Summerskill, W. H. J., Go, V. L. W. (1976) Measurement of gastric functions during digestion of ordinary solid meals in man. *Gastroenterology* **70**: 203–210

McGhee, J. R., Czerkinsky, C., Mestecky, J. (1999) Mucosal vaccines: an overview. In: Ogra, P. L., Mestecky, J., Lamm, M., Strober, W., Bienstock, J., McGhee, J. R. (eds) *Mucosal immunology*. 2nd edn, Academic Press, London, pp 741–757

Owen, R. L., Piazza, A. J., Ermak, T. H. (1991) Ultrastructural and cytoarchitectural features of lymphoreticular organs in the colon and rectum of adult Balb/c mice. *Am. J. Anat.* **190**: 10–18

Richardson, C., Walsh, J., Hicks, M., Fordtran, J. (1976) Studies on the mechanism of food simulated gastric acid secretion in normal human subjects. *J. Clin. Invest.* **58**: 623–631

Russell, T. L, Berardi, R. R, Barnett, J. K, Dermentzoglou, L. C, Jarvenpaa, K. M, Schmaltz, S. P, Dressman, J. B. (1993) Upper gastrointestinal pH in seventy-nine healthy, elderly North American men and women. *Pharm. Res.* **10**: 187–196

Schiller, C., Frohlich, C. P., Geissman, T., Siegmund, W., Monnikes, H., Hosten, N., Weitschies, W. (2005) Intestinal fluid volumes and transit of dosage forms as assessed by magnetic resonance imaging. *Aliment. Pharm. Ther.* **22**: 971–979

Smith, H. W. (1965) Observations of the flora of the alimentary tract of animals and factors influencing its composition. *J. Pathol. Bacteriol.* **89**: 95–122

Ward, F. W., Coates, M. E. (1987) Gastrointestinal pH measurement in rats: influence of the microbial flora, diet and fasting. *Lab. Anim.* **21**: 216–222

Wolfensohn, S., Lloyd, M. (1994) *Handbook of laboratory animal management*. Oxford University Press, Oxford

Wong, S. M., Kellaway, I. W., Murdan, S. (2006) Fast dissolving microparticles fail to show improved oral bioavailability. *J. Pharm. Pharmacol.* **58**: 1319–1326

Bohn, Brent

From: Gibbons, Catherine
Sent: Friday, December 04, 2015 7:26 PM
To: Bohn, Brent
Subject: FW: Cal/EPA meeting

From: Chiu, Weihsueh
Sent: Tuesday, November 25, 2014 3:36 PM
To: Gibbons, Catherine <Gibbons.Catherine@epa.gov>
Cc: D'Amico, Louis <DAmico.Louis@epa.gov>; Shams, Dahnish <Shams.Dahnish@epa.gov>; Sasso, Alan <Sasso.Alan@epa.gov>
Subject: Re: Cal/EPA meeting

Sounds great!

--Weihsueh
Please excuse terse responses. Sent from my iPhone

On Nov 25, 2014, at 3:35 PM, "Gibbons, Catherine" <Gibbons.Catherine@epa.gov> wrote:

> Hi all, Elaine Khan at Cal/EPA had some questions on PBPK and asked Alan if he could have a quick teleconference; she has invited several others there as well. I don't see a problem with this at all but I just wanted to let you know. Thanks!
>
> -----Original Appointment-----
> **From:** Khan, Elaine@OEHHA [mailto:Elaine.Khan@oehha.ca.gov]
> **Sent:** Tuesday, November 25, 2014 2:38 PM
> **To:** Khan, Elaine@OEHHA; Sasso, Alan; Gibbons, Catherine; Alexeeff, George@OEHHA; Wong, Patty@OEHHA; Ting, David@OEHHA; Hirsch, Allan@OEHHA; Zeise, Lauren@OEHHA
> **Subject:** PBPK Manuscript
> **When:** Wednesday, November 26, 2014 12:00 PM-1:00 PM (UTC-08:00) Pacific Time (US & Canada).
> **Where:** conference call
>
> (meeting time is 3:00 pm EST)
>
> **USA Toll-Free:**
>
> **Host Code:**
>
> **Participant Code:**

Bohn, Brent

From: Gibbons, Catherine
Sent: Friday, December 04, 2015 7:26 PM
To: Bohn, Brent
Subject: FW: Cal/EPA meeting

From: D'Amico, Louis
Sent: Tuesday, November 25, 2014 3:55 PM
To: Gibbons, Catherine <Gibbons.Catherine@epa.gov>; Shams, Dahnish <Shams.Dahnish@epa.gov>; Chiu, Weihsueh <Chiu.Weihsueh@epa.gov>
Cc: Sasso, Alan <Sasso.Alan@epa.gov>
Subject: RE: Cal/EPA meeting

Sounds good to me!

Louis D'Amico, Ph.D.
Acting Communications Director, ORD/NCEA
damico.louis@epa.gov
O: (703) 347-0344 M: (703) 859-1719

From: Gibbons, Catherine
Sent: Tuesday, November 25, 2014 3:35 PM
To: D'Amico, Louis; Shams, Dahnish; Chiu, Weihsueh
Cc: Sasso, Alan
Subject: Cal/EPA meeting

Hi all, Elaine Khan at Cal/EPA had some questions on PBPK and asked Alan if he could have a quick teleconference; she has invited several others there as well. I don't see a problem with this at all but I just wanted to let you know. Thanks!

-----Original Appointment-----
From: Khan, Elaine@OEHHA [mailto:Elaine.Khan@oehha.ca.gov]
Sent: Tuesday, November 25, 2014 2:38 PM
To: Khan, Elaine@OEHHA; Sasso, Alan; Gibbons, Catherine; Alexeeff, George@OEHHA; Wong, Patty@OEHHA; Ting, David@OEHHA; Hirsch, Allan@OEHHA; Zeise, Lauren@OEHHA
Subject: PBPK Manuscript
When: Wednesday, November 26, 2014 12:00 PM-1:00 PM (UTC-08:00) Pacific Time (US & Canada).
Where: conference call

(meeting time is 3:00 pm EST)

USA Toll-Free:

Host Code:

Participant Code:

Bohn, Brent

From: Gibbons, Catherine
Sent: Friday, December 04, 2015 7:25 PM
To: Bohn, Brent
Subject: FW: PBPK manuscript

From: Khan, Elaine@OEHHA [mailto:Elaine.Khan@oehha.ca.gov]
Sent: Friday, December 05, 2014 2:43 PM
To: Sasso, Alan <Sasso.Alan@epa.gov>; Gibbons, Catherine <Gibbons.Catherine@epa.gov>
Subject: RE: PBPK manuscript

Thank you, Alan!

From: Sasso, Alan [mailto:Sasso.Alan@epa.gov]
Sent: Friday, December 05, 2014 11:41 AM
To: Khan, Elaine@OEHHA; Gibbons, Catherine
Subject: RE: PBPK manuscript

Hi Elaine,

I just thought I'd send this to you. This paper just came out yesterday.

-Alan

From: Khan, Elaine@OEHHA [mailto:Elaine.Khan@oehha.ca.gov]
Sent: Tuesday, November 25, 2014 2:23 PM
To: Gibbons, Catherine; Sasso, Alan
Subject: RE: PBPK manuscript

Ok, that's perfect. Sorry to keep you guys from starting your Thanksgiving early, but duty calls! I really appreciate you guys accommodating us with this meeting! I'll send call-in info shortly. Happy Thanksgiving!

From: Gibbons, Catherine [mailto:Gibbons.Catherine@epa.gov]
Sent: Tuesday, November 25, 2014 11:20 AM
To: Khan, Elaine@OEHHA; Sasso, Alan
Subject: RE: PBPK manuscript

Hi Elaine! 3 pm works for both of us; I can't at noon (EST). I'll be calling in from home but that should work fine. Thanks!

From: Khan, Elaine@OEHHA [mailto:Elaine.Khan@oehha.ca.gov]
Sent: Tuesday, November 25, 2014 2:16 PM
To: Sasso, Alan
Cc: Gibbons, Catherine
Subject: RE: PBPK manuscript

Hi, Alan.

Looks like noon or 3:00 pm your time would work better for Lauren. Hope one of those times would work for you guys too. If not, we can keep the 1:00 pm and I'll follow up with calling info. Sorry for the change up! Thanks!
Elaine

From: Sasso, Alan [mailto:Sasso.Alan@epa.gov]
Sent: Tuesday, November 25, 2014 10:59 AM
To: Khan, Elaine@OEHHA
Cc: Gibbons, Catherine
Subject: RE: PBPK manuscript

Sure, Catherine and I are free at that time.

Just let us know the number to call.

-Alan

From: Khan, Elaine@OEHHA [mailto:Elaine.Khan@oehha.ca.gov]
Sent: Tuesday, November 25, 2014 1:49 PM
To: Sasso, Alan
Cc: Gibbons, Catherine
Subject: PBPK manuscript

Hi, Alan.
The topic of the manuscript you're currently preparing came up in one of my meetings yesterday and some folks here had some questions that I couldn't quite answer. I was wondering if you might have some time tomorrow (if you're even working) to have a short meeting to discuss the paper? If so, how does 1:00 pm your time sound? Please let me know and I'll set it up. Thanks!
Elaine

Elaine M. Khan, Ph.D.
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Bohn, Brent

From: Gibbons, Catherine
Sent: Friday, December 04, 2015 7:24 PM
To: Bohn, Brent
Subject: FW: talk about proposal

-----Original Appointment-----
From: Gibbons, Catherine
Sent: Monday, April 20, 2015 5:19 PM
To: Elaine.Khan@oehha.ca.gov
Cc: Zeise, Lauren@OEHHA; Sasso, Alan; Rieth, Susan
Subject: Accepted: talk about proposal
When: Tuesday, April 21, 2015 1:00 PM-2:00 PM (UTC-08:00) Pacific Time (US & Canada).
Where: phone

Hi Elaine, why don't we just use our conference line?

Thanks, talk to you tomorrow!

Bohn, Brent

From: Gibbons, Catherine
Sent: Friday, December 04, 2015 7:23 PM
To: Bohn, Brent
Subject: FW: SciPinion Survey from SOT 2015

From: Zeise, Lauren@OEHHA [mailto:Lauren.Zeise@oehha.ca.gov]
Sent: Tuesday, April 21, 2015 5:01 PM
To: Gibbons, Catherine <Gibbons.Catherine@epa.gov>; Elaine.Khan@oehha.ca.gov
Subject: RE: SciPinion Survey from SOT 2015

Interesting

From: Gibbons, Catherine [mailto:Gibbons.Catherine@epa.gov]
Sent: Tuesday, April 21, 2015 1:58 PM
To: Khan, Elaine@OEHHA; Zeise, Lauren@OEHHA
Subject: FW: SciPinion Survey from SOT 2015

FYI

From: Jarabek, Annie
Sent: Tuesday, April 21, 2015 1:35 PM
To: Flowers, Lynn; Jones, Samantha; Gibbons, Catherine; Sasso, Alan
Subject: SciPinion Survey from SOT 2015

Chromium Crew

Here are the questions that I used to chair the session on SciPinion at SOT in San Diego. Rita was a panel member along with Bette Meek and Rick Becker. The MOA presentation for each chemical went per usual with questions / comments after each. The Poll then followed after the "section" (i.e., chemical), followed by some general queries on SciPinion. One thing I didn't mention was that BOTH case studies sort of got sent home with lessons learned – definitely wasn't a slam dunk for either.

In this case I guess after the 2 PBPK and then another after the mutagenic/non-mutagenic. We could ask for general queries on SciPinion too which would reinforce the "we are learning what this tool provides" context. Worth a try?

Let me know what you decide?

Annie

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Go Green - print this email only if necessary

Memorandum

To: Ambuja Bale, Hui-Min Yang

From: Julie Stickney, Heather Carlson-Lynch

Date: July 22, 2009

Re: Foreign language studies of n-butanol

This memorandum is intended to inform EPA of several n-butanol studies published in foreign languages so that EPA can consider whether to translate the papers. In addition, SRC would like to note that the RTI (1985) oral subchronic rat study provided to SRC is missing data tables.

A number of foreign language references were identified by tree-searching reference lists during preparation of the draft report for n-butanol. Below is a list of acute-duration animal studies published in languages other than English, and summaries of subchronic, chronic or reproductive/developmental studies that are only available in foreign languages. Because several other acute-duration animal studies are available, SRC does not recommend translation of the acute studies. Brief information on the acute-duration foreign language studies (based on secondary sources) will be added to the report.

Several of the longer term studies are also of limited utility. Of the longer term studies, only Bariliak et al. (1991) appears to be of adequate quality to consider translation. SRC seeks EPA technical direction on the use of the foreign language papers shown herein. Pending further instruction, SRC will use the summaries provided below (derived from secondary source information) in the toxicological review as placeholders.

Acute Duration Animal Studies:

EGOROV, Y.L. (1972) Dependance of dermal toxicity of alcohols on solubility index: oil/water. *Toksikol. Gig. Prod. Neftekhim Yarosl.*, **98**: 102.

DUBINA, O.N. & MAKSIMOV, G.G. (1976) [Testing the use of golden hamsters in toxicological research.] *Gig. Tr. Ohkhr. Zdorov'ya Rab. Neft. Neftekhim. Prom-sti,* **9**: 100-103 (in Russian).

RUMYANSTEV, A.P., LOBANOVA, I.YA., TIUNOVA, L.V., & CHERNIKOVA, V.V. (1979) [Toxicology of butyl alcohol.] *Khim. Prom.-st. Ser. Toksikol. Sanit. Khim. Plastmass,* **2**: 24-26 (in Russian).

LENDLE, L. (1928) [Investigations on the speed at which homologous and isomeric monovalent alcohols produce narcosis.] *Naunyn-Schmiedeberg's Arch. exp. Pathol. Pharmakol.,* **129**: 85 (in German).

SAITO, M. (1975) [Studies on the metabolism of lower alcohols.] *Nichidai Igaku Zasshi,* **34**(8-9): 569-585 (in Japanese).

Subchronic, chronic or reproductive/developmental studies:

BAIKOV, B.K. & KHACHATURYAN, M.Kh. (1973) [Hygienic assessment of the reflex action on a body of small concentrations of butyl alcohol in the atmosphere.] *Gig. i Sanit.,* **12**: 7-11 (in Russian).

Bariliak, IR; Korkach, VI; Spitkovskaia, LD. (1991) [The embroyotoxic effects of monohydric alcohols] (Russian). 22(1):71-75.

SAVELEV, A.I., BABANOV, A.G., SKOBEI, N.A., & TROITSKAYA, I.A. (1975) [Adaptation reactions of white rats after prolonged administration of small concentrations of butyl alcohol.] In: Zaikina, M.G., ed. *[Pathophysiology of the cardiovascular system,]* Yaroslav, Yaroslav Medical Institute, pp. 59-62, 76-80 (in Russian).

Seitz, B. (1972) [Occurrence of serious vertigo after handling of butanol and isobutanol: three cases] (French). Archives Mal Prof Med Trav Secur Soc 33:393-395.

KOLESNIKOV, P.A. (1975) [Adaptation to butyl alcohol.] *Gig i Sanit.,* **(5)**: 104-105 (in Russian).

RUMYANSTEV, A.P., LOBANOVA, I.YA., TIUNOVA, L.V., & CHERNIKOVA, V.V. (1979) [Toxicology of butyl alcohol.] *Khim. Prom.-st. Ser. Toksikol. Sanit. Khim. Plastmass,* **2**: 24-26 (in Russian).

Summary of foreign subchronic, chronic or reproductive/developmental studies:

Seitz (1972) (published in French, no abstract, limited translation completed by SRC) described several cases of vertigo in laboratory employees exposed to n-butanol and/or isobutanol vapors. A total of seven cases were described briefly; three cases were reported to have exposure to n-butanol and the remainder to isobutanol. Of the three exposed to n-butanol, two reported no symptoms; one was a chemist exposed approximately one-half day per week for one month, and

the other had been exposed for less than a year (frequency not reported). The third was a photographer who was exposed to n-butanol approximately two to three hours per day in a dark room over the course of one year. This subject reported stomach symptoms (nausea and bloating) as well as vertigo. He was ultimately diagnosed with Ménière's-type vertigo. No other information (e.g., exposure levels or follow-up information) was given in the report.

Bariliak et al.(1991) was published in Russian with an English summary (no study details). A detailed summary of this reports was provided in the Cosmetic Ingredient Review Expert Panel report on n-butanol (McLain, 2008). According to (McLain, 2008), groups 10-16 white rats (weighing 160-180 g, strain not specified) were given various alcohols (methanol, ethanol, n-butanol, nonanol, and decanol; purity not given) by gavage (1 mL of 40% solution in water) from GD 1 through 15. Without information on the body weights of the pregnant rats, estimating a dose associated with the administered solution of n-butanol is difficult. Assuming a body weight of 0.250 kg, a dose of about 1600 mg/kg-day would be estimated. However, the estimated dose is highly uncertain. Controls (20 rats) were given water alone. At sacrifice on GD 20, the numbers of corpora lutea and live and dead fetuses were counted. The review did not discuss any maternal evaluations. Toxicological evaluations reported in the secondary source include: fertility index (description not provided), number of implantations, percent pre- and postimplantation losses, and number of live fetuses. In addition, alcohol dehydrogenase activity was measured in livers excised from selected fetuses (1-2/litter). According to (McLain, 2008), these measurements were performed daily on fetuses from GD 16-21 and on PND 1, 3, and 20; it is not clear whether separate groups of animals were used for these assessments. According to the review, treatment with n-butanol resulted in significant ($p<0.001$) increases in the percents of pre- and postimplantation losses and in total fetal deaths. A decrease in the fertility index of treated animals was reported in the review (6.5% vs. 9.7% in controls; statistical analysis not reported). In addition, ADH activity in fetal livers, which was at its highest level on GD 20, was reduced 77.6% at this measurement in offspring of dams exposed to n-butanol. No other information was provided in the review or in the English summary in the publication.

Several secondary sources reviewed a group of studies published in Russian, most without English abstracts or summaries. Information on study design and findings is available from the

secondary sources. None of the secondary sources reported details of exposure chambers, vapor generation systems, animal strains or sexes tested, number of animals tested per group, presence or absence of untreated or sham-treated control groups, toxicological evaluations, or in most cases effects associated with specific concentrations. According to the reviews, Baikov and Khachaturyan (1973) exposed rats to 0.09 or 21.8 mg/m^3 n-butanol (0.03 or 7.1 ppm) continuously for 92 days (reviewed by WHO, 1987 and MOE, 2007). WHO (1987) and MOE (2007) reported that exposure to 0.09 mg/m^3 was without effect. At 21.8 mg/m^3 n-butanol, effects included decreased RNA and DNA in blood, increased leukocyte luminescence, increased diastase activity, decreased catalase activity, and increased transport of n-butanol across the blood:tissue barriers in the testis, spleen, and thyroid (WHO, 1987; MOE, 2007).

Savalev et al. (1975) exposed rats to 218 mg/m^3 n-butanol (71 ppm) for five hours/day, six days/week, for six months (reviewed by WHO, 1987 and MOE, 2007). Effects noted in the treated animals during the first two months included decreased oxygen consumption and delay in restoration of normal body temperature after cooling. During the following four months, these effects were resolved (Savalev et al., 1975, as reviewed by WHO, 1987 and MOE, 2007).

Kolesnikov (1975) exposed mice continuously for 30 days to concentrations of 13.6 or 40.01 mg/m^3 (2.1 or 13 ppm) (reviewed by WHO, 1987 and MOE, 2007). Decreased sleeping time was reported as the only effect (Kolesnikov, 1975, as reviewed by WHO, 1987).

Rumyantsev et al. (1979) exposed albino rats and mice to butanol via inhalation at concentrations of 0.8, 6.6, or 40 mg/m^3 24 hours/day for four months (reviewed by WHO, 1987 and MOE, 2007). In the English summary, the study authors reported that the low concentration (0.8 mg/m3), while "effective," resulted in "no pathological shifts in vital functions of the body", while concentrations of 6.6 and 40 mg/m^3 had "an unfavorable effect on experimental animals". The study authors recommended an "ineffective concentration" of 0.1 mg/m^3 for 24-hour exposure. WHO (1987) reported the following effects of treatment: "decreased sleeping time; stimulated blood cholinesterase; disturbances of reflexes and neuromuscular sensitivity of the nervous system; increased thyroid activity and secretion of thyroxine; increases in eosinophile leukocytes in blood after injection of adreno-corticotrophin (ACTH)". WHO (1987) did not

specify the concentrations at which these effects were observed; MOE (2007) reported that these effects occurred at 40 mg/m^3.

Bohn, Brent

From: Khan, Elaine@OEHHA <Elaine.Khan@oehha.ca.gov>
Sent: Tuesday, July 23, 2013 4:04 PM
To: Sasso, Alan
Subject: Automatic reply: PBPK model errata for hexavalent chromium

I will be out of the office from July 22nd through July 26th. I will respond to email when I return on July 29th. If you need immediate assistance, please contact Dr. Patty Wong (patty.wong@oehha.ca.gov).

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носила субъективный характер. Настоящий метод, по-видимому, можно использовать в комплексе с другими и чаще в виде вспомогательного метода.

Основываясь на имеющемся реальном опыте, можно рекомендовать комплексное использование перечисленных выше прогностических методов и следующую последовательность действий при прогнозировании в гигиене.

1. Разработка задания на прогноз. Ответственные за разработку прогноза организации и лица должны определить объект прогнозирования (широту охвата его), указать время упреждения прогностических оценок и формы использования результатов прогноза.

2. Предпрогностическая ориентировка. Уяснение рабочих деталей задания на прогноз: организация сбора необходимой для работы информации; предварительное тщательное ознакомление с имеющимися научными обзорами, литературными данными и прогностическими оценками, относящимися к данному объекту прогнозирования; составление максимально детализированного плана работы по прогнозированию. Тщательно продуманный план работы является залогом высококачественного прогноза.

3. Анализ тенденций и оценка уровня научно-технического развития. С этого этапа начинается собственно прогностическая деятельность. Подготовляется синтетический обзор тенденций развития в прошлом и настоящем. Весьма важным является сравнительная оценка уровня научно-технического развития гигиены в различных странах мира.

4. Построение матрицы «цель — средства». На этом этапе предлагаются по возможности объективные и логически непротиворечивые формулировки генеральной цели развития прогнозируемого объекта, основных подцелей конкретных путей, ведущих к генеральной цели, арсенала принципиально возможных направлений научно-технических работ, которые могут послужить средством достижения тех или иных подцелей, а тем самым и содействовать достижению генеральной цели. Рекомендуется составление специальной матрицы, клетки которой в дальнейшем заполняются сведениями и оценками соответствующих «весовых» коэффициентов вероятностей событий. Этот этап имеет чрезвычайно важное значение для успеха всей дальнейшей работы.

5. Выявление и анализ возможностей использования научно-технического прогресса. Осуществление ранжировки полученного списка ключевых событий по их относительной значимости для изменения санитарной ситуации и развития гигиенической науки.

6. Формулировка возможной последовательности тех или иных групп и ключевых событий, увязка их в гипотетические сети программ работ. Важная задача этого этапа работ — вероятностная оценка меры реальности и предполагаемых сроков осуществления прогнозируемых событий.

7. Определение требований, оценка последствий и перспектив развития гигиенической науки в связи с результатами предыдущего этапа работ. Важным при этом является укрупненная оценка ресурсов, необходимых для достижения различными путями тех или иных целей научно-технического развития. На этой стадии прогнозы развиваются в «организационный» прогноз, существенная часть которого — решение задачи оптимального обеспечения и распределения ресурсов между различными прогнозируемыми направлениями.

8. Формулировка комплексной концентрации научно-технического развития гигиены в виде системы аргументированных положений и определенных показателей и параметров. Изложение этой концентрации дополняется описательным документом типа «сценария будущего», в котором наряду с предполагаемой стратегической доктриной развития гигиенической науки, формулируются наиболее перспективные с точки зрения прогностических данных направления развития ряда смежных отраслей науки, техники и градостроительства.

Прогнозирование гигиены, как науки, развитие которой может быть достоверно предсказано только при обстоятельном учете тенденций и перспектив развития смежных отраслей науки и прогноза развития всего народного хозяйства в целом, как фактора, определяющего состояние окружающей среды на рассматриваемый период времени, представляет чрезвычайно трудную и очень ответственную задачу, поэтому оно должно производиться с учетом предшествующего опыта прогнозирования в гигиене, недостатков в его проведении, а также использования всего теоретического багажа знаний, накопленного при проведении прогностических работ в нашей стране и за рубежом. Достоверные научно обоснованные гигиенические прогнозы изменения окружающей среды в населенных местах СССР являются основой для прогнозирования важнейших направлений гигиенических исследований и для прогнозирования и планирования государственных оздоровительных мероприятий.

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УДК 614.72:547.364

Кандидаты мед. наук *Б. К. Байков, М. Х. Хачатурян*

ГИГИЕНИЧЕСКАЯ ОЦЕНКА РЕФЛЕКТОРНОГО ДЕЙСТВИЯ НА ОРГАНИЗМ МАЛЫХ КОНЦЕНТРАЦИЙ БУТИЛОВОГО СПИРТА, ПОСТУПАЮЩЕГО В АТМОСФЕРНЫЙ ВОЗДУХ

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Низшие спирты являются соединениями, широко используемыми в производстве моющих средств в качестве присадок к смазочным маслам, флотореагентов, в лакокрасочной, парфюмерной, фармацевтической и других отраслях промышленности. Будучи весьма летучими веществами, они могут распространяться внутри производственных помещений и поступать в атмосферный воздух вместе с промышленными выбросами.

Среди низкоатомных спиртов, загрязняющих воздушный бассейн, видное место занимает бутиловый спирт. Ввиду высокой реакционной способности бутанола как типичного представителя спиртов, а следовательно, и возможной его высокой токсичности мы поставили своей задачей изучить кратковременное действие малых концентраций бутилового спирта с целью установления его максимально разовой предельно допустимой концентрации в атмосферном воздухе.

Порог запаха бутилового спирта определен общепринятым методом (В. А. Рязанов и соавт.) на 18 практически здоровых наблюдаемых в возрасте от 18 до 45 лет. Изучение 5 концентраций (от 15 до 0,9 *мг/м*³) показало, что минимально ощутимая концентрация бутилового спирта для наиболее чувствительных лиц равна 1,2 *мг/м*³, а максимально неощутимая — 0,9 *мг/м*³. Иначе говоря, пороговой по ощущению запаха является концентрация бутанола, равная 1,2 *мг/м*³.

Известно, что порог ощущения запаха не служит пределом физиологической активности токсических веществ. Поэтому отсутсвие запаха еще не свидетельствует о том, что под влиянием неощутимых концентраций в организме не возникают рефлекторные реакции с рецепторов органов дыхания. Для их выявления используют различные методы.

Порог рефлекторного действия бутилового спирта на световую чувствительность темноадаптированного глаза определялся с помощью адаптометра марки АДМ на 3 испытуемых в возрасте 24—28 лет, ощущавших запах бутилового спирта в концентрациях 1,1—1,2 *мг/м*³. Сначала у всех наблюдаемых в течение нескольких дней регистрировали кривую темновой адаптации при дыхании чистым воздухом. Затем с 15-й по 20-ю минуту в воздух добавляли бутиловый спирт в изучаемой концентрации.

Результаты темновой адаптации глаза, изучаемой у наблюдаемой Т. Б., представлены на рис. 1. Бутиловый спирт в концентрации 1,2 *мг/м³* ведет к замедлению темновой адаптации. До конца обследования световая чувствительность намного ниже, чем в фоновых опытах. Концентрация бутилового спирта, равная 0,9 *мг/м³*, не вызывает заметных изменений кривой темновой адаптации. Аналогичные результаты зарегистрированы и у 2 других наблюдаемых.

Кроме того, для выявления действия на организм субсенсорных, по ощущению запаха, концентраций бутилового спирта мы изучили скрытое время условнорефлекторной двигательной реакции на свет. В. В. Закусов, С. И. Горшков, Е. И. Бойко и др. доказали, что скрытое время двигательной реакции меняется в результате воздействия на организм фармакологических и токсических веществ, факторов внешней среды, при патологических состояниях центральной нервной системы. Установлено, что эта реакция является показателем лабильности центральной нервной системы.

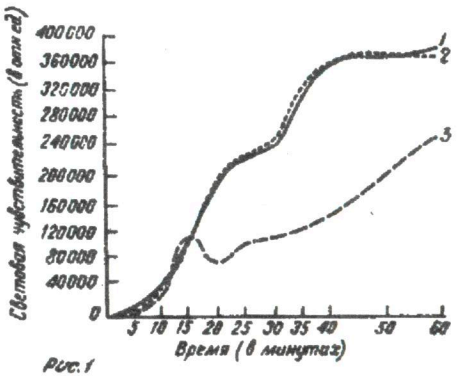

Рис. 1

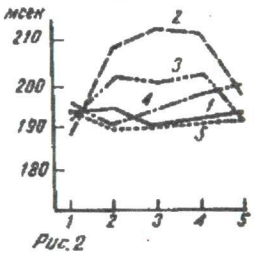

Рис. 2

Рис. 1. Кривая темновой адаптации глаза у наблюдаемой Т. Б. (*1*) и ее изменение под влиянием паров бутилового спирта в концентрациях 1,2 *мг/м³* (2) и 0,9 *мг/м³* (*3*).

Рис. 2. Влияние малых концентраций бутилового спирта на скрытое время условнорефлекторной двигательной реакции на свет у наблюдаемого И. П.

1 — чистый воздух; *2* — концентрация бутилового спирта 2,5 *мг/м³*; *3* — концентрация бутилового спирта 1,5 *мг/м³*; *4* — концентрация бутилового спирта 0,7 *мг/м³*; *5* — концентрация бутилового спирта 0,5 *мг/м³*.

Исследование проведено нами по методике М. Х. Хачатурян и соавт. у 3 наблюдаемых (Р. К., И. П. и С. В.), для которых порог ощущения запаха бутилового спирта соответственно равен 2,5, 1,5 и 1,2 *мг/м³*.

Скрытое время двигательной реакции, зарегистрированное у наблюдаемого И. П., приведено на рис. 2. Бутиловый спирт в концентрациях 2,5 и 1,5 *мг/м³* приводит к увеличению этого времени, а концентрация 0,7 *мг/м³* оказывает двухфазное действие. Аналогичные результаты зафиксированы у наблюдаемого С. В. Статистическая обработка полученных данных показала, что бутиловый спирт в концентрации 0,7 *мг/м³* приводит к достоверным сдвигам скрытого времени реакции; концентрация 0,5 *мг/м³* оказалась недействующей.

У наблюдаемой К. Р. (порог ощущения запаха 2,5 *мг/м³*) бутиловый спирт в концентрации 0,7 *мг/м³* не вызывал статистически достоверных сдвигов.

Таким образом, скрытое время условнорефлекторной реакции более чувствительно к действию низких концентраций бутилового спирта, чем световая чувствительность глаза; выявлено действие субсенсорных концентраций бутилового спирта. Такие результаты позволяли думать, что условнорефлекторная реакция более чувствительна к изучаемому воздействию, чем безусловнорефлекторная (темновая адаптация глаза).

Многочисленные исследования высшей нервной деятельности, проведенные на животных и человеке, свидетельствуют о том, что тормозной процесс более хрупкий, чем процесс возбуждения. Изучению его посвящена следующая серия наших исследований.

Уже в первые годы изучения высшей нервной деятельности в лабораториях И. П. Павлова возник вопрос о возможности образования условных рефлексов высокого порядка, т. е. таких, которые вырабатываются при подкреплении индифферентного агента не безусловным, а условным раздражителем. Оказалось, что присоединяемый агент не только не вызывал условной реакции, свойственной раздражителю первого порядка, но даже тормозил ее. Поэтому он получил название условного тормоза. Условное торможение изучалось многими исследователями, однако никем не было предпринято попытки выработать условный тормоз из подпорогового по ощущению раздражения.

В ходе каждого нашего обследования условный световой раздражитель, вызывавший двигательную реакцию наблюдаемого, применялся 10 раз

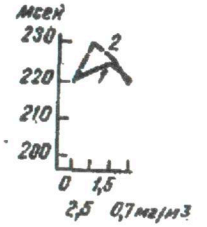

Рис. 3. Изменение среднего скрытого времени двигательной реакции на свет под влиянием следового условного тормоза
На оси абсцисс — интенсивность обонятельного раздражения (в микрограммах бутилового спирта на 1 *м³*); на оси ординат — скрытое время условной двигательной реакции на свет; *1* — изменения у наблюдаемой Б; *2* — изменения у наблюдаемой Ж.

спаузами от 30 до 60 сек. В ходе выработки условного тормоза световому раздражителю предшествовало 10-секундное действие запаха при паузе между раздражителями, равной 1 сек. В остальном сохранялись условия, имевшие место в предыдущей серии эксперимента. При подключении газа дополнительной инструкции наблюдаемый не получал. Исследования проведены на 3 наблюдаемых (Ж., Б. и Н.); порог запаха бутилового спирта был соответственно равен 1, 2, 1,5 и 2 *мг/м³*. Выработка условного тормоза началась с запаха бутилового спирта в концентрации 2,5 *мг/м³*. В ходе выработки условного тормоза наблюдались все 3 известные фазы — внешнее торможение, отсутствие действия прибавочного агента и фаза условного торможения. Их выраженность у разных испытуемых была неодинаковой и зависела от относительной силы прибавочного агента.

Среднее скрытое время условной двигательной реакции и ее изменение под влиянием условного тормоза разной интенсивности показаны на рис. 3. Бутиловый спирт в концентрациях 2,5 и 1,5 *мг/м³* оказал статистически достоверное тормозное действие на условный двигательный рефлекс. Концентрация бутилового спирта, равная 0,7 *мг/м³*, не вызывала достоверного тормозного действия. Таким образом, нам удалось у 2 наблюдаемых выявить действие бутилового спирта на уровне порога запаха, а у 1 наблюдаемой (Н.) — в подпороговой по ощущению запаха концентрации.

Последним этапом нашей работы явилось изучение действия малых концентраций бутилового спирта на электрическую активность коры головного мозга. Использован метод взаимодействия условного и безусловного раздражителей (М. Х. Хачатурян и В. М. Стяжкин). Эти исследователи, а также Ф. И. Дубровская и соавт., О. Е. Горлова показали, что действие подпороговых по ощущению запаха концентраций газа лучше выявляется в теменном и затылочном отведениях. Кроме того, данные Ф. И. Дубровской и соавт., а также О. Е. Горловой, свидетельствовали о преимуществе дробной регистрации реакции на световой раздражитель.

Это было использовано нами в исследовании 3 наблюдаемых в возрасте 18—28 лет. Во время обследований они с закрытыми глазами полулежали в кресле в темной, звукозаглушенной, экранированной камере. Перед лицом наблюдаемых находился нюхательный цилиндр с рассеивателем, через который подавался чистый воздух или газовая смесь со ско-

ростью 25 *л* в минуту. Момент переключения наблюдаемыми не ощущался. Регистрация энцефалограммы и ее анализ осуществлялись комплексом приборов фирмы «Orion». Условным раздражителем служили пары бутилового спирта в концентрациях 1,5, 0,7, 0,3, 0,2 и 0,1 *мг/м*³. В качестве безусловного раздражителя использован ритмический свет с частотой, оптимально усваиваемой каждым наблюдаемым.

У 2 наблюдаемых можно было отметить образование условного рефлекса, да и то в виде нестойкого переходящего явления. У всех испытуемых условный раздражитель менял течение безусловного рефлекса. Недействующей во всех случаях оказалась концентрация бутилового спирта, равная 0,1 *мг/м*³.

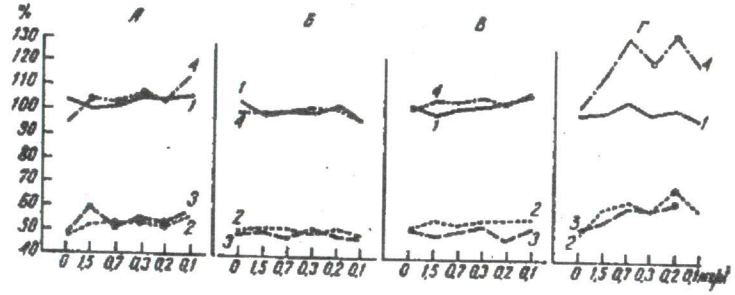

Рис. 4. Изменения электрической активности коры головного мозга у наблюдаемого И. П. в ходе выработки условного рефлекса и влияние условного раздражителя на безусловную реакцию.

На оси абсцисс — концентрация бутилового спирта (в *мг/м*³); на оси ординат — изменение активности при действии газа (в % к фону): *1* — действие газа; *2* — первые 5 сек. действия газа; *3* — вторые 5 сек. действия газа; *4* — суммарная реакция на световой раздражитель. Суммарная энцефалограмма теменной (*А*) и затылочной (*Б*) областей коры головного мозга и выделенный из нее тета-ритм теменной (*В*) и затылочной (*Г*) областей.

В качестве примера на рис. 4 приведены результаты исследований наблюдаемого И. П. Порог ощущения запаха бутилового спирта для него равен 1,2 *мг/м*³, оптимальная частота световых мельканий — 5 *гц*. Как видно из графиков, в ходе выработки условного рефлекса происходят сдвиги в суммарной ЭЭГ теменной области (*а*), суммарной ЭЭГ затылочной области (*в*) и наиболее выраженные по диапазону тэта-ритма затылочной области (*г*).

Выводы

1. Исследования атмосферы вокруг химического комбината показали, что она загрязнена промышленными выбросами, содержащими пары бутилового спирта.

2. Исследуя темновую адаптацию глаза, хронорефлексометрию, электрическую активность коры головного мозга, мы установили, что бутиловый спирт в пороговых и субсенсорных концентрациях меняет течение изученных показателей. Недействующей во всех случаях оказалась концентрация бутанола, равная 0,1 *мг/м*³. Она утверждена в качестве максимальной разовой предельно допустимой концентрации бутилового спирта в атмосферном воздухе.

ЛИТЕРАТУРА. Бойко Е. И. Время реакции человека. М., 1964.— Горлова О. Е. Гигиеническая оценка загрязнения атмосферного воздуха при производстве изопропанола и сравнительная характеристика действия пропилового и изопропилового спиртов на организм. Дисс. канд. М., 1969.— Горшков С. П. Скрытое время рефлекторных реакций как адекватный показатель функционального состояния нервной системы. Дисс. докт. М., 1962.— Дубровская Ф. П. и др. В кн.: Материалы Республиканск. научной конференции по итогам гигиенических исследований за 1966—1967 г. Ставрополь, 1969, с. 17.— Закусов В. В. Экспериментальные данные по фармакологии центральной нервной системы. Л., 1947, с. 7.— Рязанов В. А., Бушуева К. А., Новиков Ю. В. В кн.: Предельно допустимые концентрации атмосферных загрязнений. М., 1957, в. 3, с. 117.— Хачатурян М. Х., Гусев М. И., Горлова О. Е. Гиг. и сан., 1968, № 8, с. 101.— Хачатурян М. Х., Стяжкин В. М. В кн.: Вопросы гигиены атмосферного воздуха и планировки населенных мест. М., 1968, с. 34.

Поступила 27/VI 1973 г.

HYGIENIC ASSESSMENT OF THE REFLEX ACTION ON A BODY OF SMALL CONCENTRATIONS OF BUTYL ALCOHOL IN THE ATMOSPHERE

B. K. Baikov, M. Kh. Khachaturyan

A study of the light sensitivity of an eye adapted to darkness revealed the reflex action of butyl alcohol at a level of its threshold value of smell. Meanwhile the conditioned reflex and the encephalographic reactions changed under the action of sensory concentrations. On the basis of investigations performed the authors recommend maximum single time permissible concentration of butyl alcohol in the atmosphere to be set at a level of 0,1 mg/m³.

УДК 614.71/.73-07:616.831-092.

Проф. *А. И. Бокина, Н. Д. Экслер*

ЭЛЕКТРОФИЗИОЛОГИЧЕСКИЙ АНАЛИЗ ДЕЙСТВИЯ НЕКОТОРЫХ АТМОСФЕРНЫХ ЗАГРЯЗНИТЕЛЕЙ НА ЦЕНТРАЛЬНУЮ НЕРВНУЮ СИСТЕМУ

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Мы попытались подойти к решению вопроса о критериях неблагоприятного воздействия токсических веществ на центральную нервную систему на примере продуктов фотохимических реакций в атмосферном воздухе — озона и формальдегида. Для выяснения функциональных сдвигов в центральной нервной системе экспериментальных животных под воздействием высоких и низких концентраций указанных токсических веществ был избран метод изучения суммарной электрической активности ряда структур мозга, обеспечивающих сенсорный ответ на применяемые запаховые раздражители (обонятельная луковица, пириформная кора), и образований, осуществляющих реакции адаптивно-поведенческого характера (гиппокамп, миндалина, ретикулярная формация ствола мозга), анализ реакции перестройки ритма в ответ на ритмическое световое раздражение и метод вызванных потенциалов (анализ комплекса первичный ответ — медленная отрицательная волна — поздний ответ).

Биоэлектрическую активность структур головного мозга регистрировали с помощью 16-канального электроэнцефалографа фирмы «Галилео». Запись вызванных потенциалов вели на двухлучевом универсальном индикаторе «Диза». Бинокулярную световую стимуляцию осуществляли серией апериодических или ритмических коротких вспышек (энергия вспышки 1,4 *дж*, длительность засвета 1,2 *мсек.*).

Формальдегид дозировали по общепринятой схеме (В. А. Рязанов и соавт.). Постоянство исследуемых концентраций контролировали при помощи методики М. В. Алексеевой с хромотроповой кислотой. Озон получали из баллонного кислорода, используя озонатор типа ОВ-1. Постоянство концентраций контролировали нейтральным йодидным методом.

На 47 кроликах поставлено около 500 опытов. После окончания опытов проводили морфологический контроль за положением электродов в изучаемых структурах мозга.

Изучение экстренного применения формальдегида и озона в опытах I серии при 10-секундной экспозиции показало, что оба вещества вызывают в зависимости от концентрации два вида реакции в центральной нерв-

Bohn, Brent

From: Khan, Elaine@OEHHA <Elaine.Khan@oehha.ca.gov>
Sent: Thursday, August 22, 2013 2:53 PM
To: Gibbons, Catherine
Cc: Sasso, Alan
Subject: CA Cr6 MCL

Hi, Catherine and Alan.

Fyi, California is releasing a proposed Cr6 MCL (10 ppb) for public comment.
http://www.cdph.ca.gov/certlic/drinkingwater/Pages/Chromium6.aspx

Elaine

Bohn, Brent

From: Sasso, Alan
Sent: Monday, March 30, 2015 8:52 AM
To: 'Wong, Patty@OEHHA'
Cc: Elaine.Khan@oehha.ca.gov; Gibbons, Catherine
Subject: copy of SOT poster
Attachments: Sasso_SOT2015.pdf

Hi Patty,

It was nice meeting you at SOT. Here is a copy of my poster from SOT.

Sorry I couldn't find anybody else from your office—SOT is very overwhelming. It's become too big. I spend most of my time there walking miles between posters and talks.

The moral of the story of this poster is that you can obtain the same human equivalent dose using a stomach-only model, as you do with a whole-body PBPK. I believe whole-body PBPK is overkill for this type of problem, and site-specific absorption rates into each GI tissue cannot be validated.

It also shows how high pH individuals may be more susceptible. We don't know the incidence of high stomach pH in the population (since it's an invasive test, usually only done when people are having GI problems).

-Alan

Alan F. Sasso, Ph.D.
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(703)-347-0179

In vivo efficiencies of hexavalent chromium reduction in the gastric environments of mice, rats, and humans

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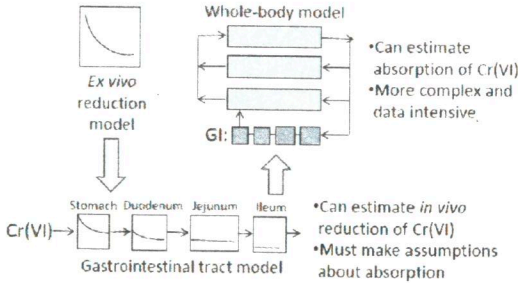
Introduction

Hexavalent chromium (Cr(VI)) is a known human carcinogen via inhalation, but less is known about human risks via ingestion. Increased incidences of neoplasms in the oral cavity of rats and in the small intestine of mice have been observed in long-term drinking water bioassays (NTP, 2008). When ingested, Cr(VI) can be reduced to trivalent chromium (Cr(III)) within the gastrointestinal (GI) tract. Cr(III) is thought to pose little or no carcinogenic risk. Understanding GI tract reduction is important in evaluating the NTP cancer findings in the context of human health risk assessment.

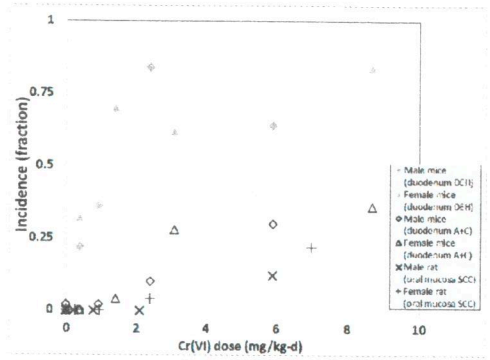
Available toxicokinetic models



Ex vivo models predict reduction under batch conditions. GI tract models incorporate ex vivo models into a dynamic system to estimate in vivo Cr(VI) reduction in the lumen. PBPK models estimate absorption and kinetics of Cr(VI) and Cr(III) in the whole body. The GI tract model in this poster combines the ex vivo model by Schlosser and Sasso (2014) with the stomach compartment of the PBPK model by Kirman et al. (2013).

Toxicity data

NTP (2008) observed diffuse epithelial hyperplasia (DEH) and adenomas and carcinomas (A+C) in the small intestine of mice. The same effects were not observed in rats, although squamous cell carcinomas (SCC) were observed in the oral mucosa.



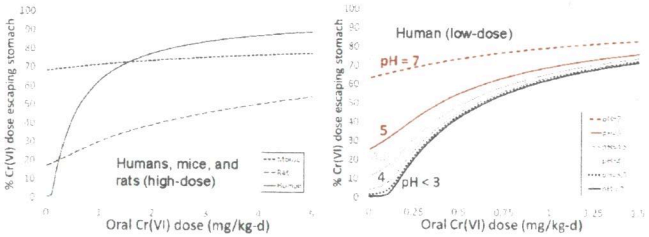
U.S. Environmental Protection Agency
Office of Research and Development

In vivo stomach reduction and GI effects

Reduction of Cr(VI) in the stomach is a major source of inter-species differences. Inefficient reduction results in a greater amount of unreduced Cr(VI) persisting in the small intestinal (SI) compartments (duodenum, jejunum, and ileum).

Species comparison of in vivo stomach reduction

- Toxicokinetic models estimate that rats reduce an equivalent daily Cr(VI) dose more efficiently than mice (on a basis of % unreduced dose escaping stomach).
- At low doses, humans reduce Cr(VI) more efficiently in the stomach than rodents, primarily due to larger stomach size, and lower pH of the human stomach.
- The high efficiency of reduction for humans also leads to a more rapid loss of reducing agent at high doses.



Two potential internal dose metrics for GI tract toxicity are:

1) **absorption** (mg Cr(VI) absorbed per L small intestine tissue), and

2) **pyloric flux** (mg Cr(VI) escaping stomach reduction, per L small intestine tissue). Pyloric flux requires only a GI tract model, while absorption requires a whole body PBPK model.

Despite the relative simplicity of the GI tract model, extrapolating NTP (2008) small intestine toxicity data from mice to humans (using **pyloric flux**) produces similar results as a whole body PBPK model (using **absorption**).

- The averages of the HEDs estimated by a GI tract model range from 0.05–0.1 mg/kg-d, depending on response rate and uncertainty factor (Table 1).
- The human equivalent dose (HED) estimated by a PBPK model was 0.06 mg/kg-d (Thompson et al., 2014).

Table 1. Preliminary dose-response and human extrapolation for diffuse epithelial hyperplasia in mice (using NTP 2008 data)

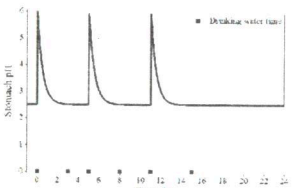
	Pyloric flux (lifetime average mg/L-d)	UF*	Adjusted pyloric flux (mg/L-d)	HED pH=2.5 (mg/kg-d)	HED pH=5 (mg/kg-d)	Average HED (mg/kg-d)
	Mouse simulations			Human simulations		
	Male mouse†					
BMDL5	1.3	3	0.43	0.12	1.4e-2	6.7e-2
BMDL10	2.6	3	0.88	0.14	2.8e-2	8.4e-2
	Female mouse‡					
LOAEL	4.0	30	0.13	8.9e-2	4.5e-3	4.7e-2
		10	0.40	0.12	1.3e-2	6.7e-2
		3	1.3	0.15	4.2e-2	9.6e-2

*Uncertainty factors.
†Male mouse internal dose BMDLs adjusted by UF of 3 to compare with Thompson et al. (2014).
‡Data for the female mouse not amenable to BMD modeling. LOAEL was adjusted by UF of 3, 10, and 30 to account for LOAEL to NOAEL extrapolation and interspecies variation. These UF values were applied for model evaluation purposes only and do not reflect an evaluation of the toxicology data for the mouse.

Benchmark dose (BMD): Dose producing a predetermined change in response rate of effect.
BMDL: Lower confidence limit on the dose at the BMD
LOAEL: Lowest observed adverse effect level

Food effects on Cr(VI) reduction

The pH of human gastric juice spikes to 6 during meals, and returns to baseline within 2 hours (Mudie et al., 2010, Parrott et al., 2009). Simulations were performed that incorporated these pH spikes, as well as changes in gastric emptying rate (KLSD) to assess the impact on average daily internal dose.



Simulation of dietary spikes in stomach pH. It was assumed that the 3 largest drinking water events were associated with a meal.

Table 2. Preliminary human extrapolation for diffuse epithelial hyperplasia in mice

Pyloric flux (mg/L-d)	flux/UF (mg/L-d)	HED (mg/kg-d)			
		pH=2.5	pH=4.9‡ KLSD=1	pH↑↓	pH↑↓ KLSD↓
4.0	0.40*	0.12	2.0e-2	1.8e-2	7.2e-2
	0.13†	8.9e-2	6.9e-3	6.2e-3	2.5e-2

*UF=10; †UF=30 (for evaluation only; see Table 1)
‡Default constant GastroPlus™ model parameters for fed human.
pH↑↓: Dynamic human pH (baseline of 2.5, spikes to 6 with meals).
KLSD↓: Constant low stomach emptying rate (KLSD=0.415/hr).

Model predictions for fed humans have higher uncertainty, and may overestimate the human internal dose because:

- Kinetic model was based on parameters derived from fasted human gastric samples
- Gastric juice of humans in the fed state will have higher reducing capacity
- Gastric emptying rate decreases during solid meals (increasing stomach reduction)

However, both the decreased reduction rate and decreased stomach emptying may increase Cr(VI) exposure to the stomach epithelium.

Discussion

- A simple 1-compartment GI tract model predicts HEDs similar to those obtained with more complex PBPK models for the endpoint of diffuse epithelial hyperplasia in mice.
- Reduction efficiencies predicted by GI tract models are consistent with NTP (2008)
  - Mice are more susceptible than rats to effects in the small intestine.
- Net effect of fed status on Cr(VI) stomach kinetics in humans is unknown.
- Analyses incorporating population variability and uncertainty of human and rodent gastric parameters is the subject of ongoing work.

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Disclaimer: The views expressed in this poster are those of the authors and do not necessarily represent the views or policies of the U.S. Environmental Protection Agency, nor does mention of trade names constitute endorsement or recommendation for use.

References

Mudie et al., (2014). Quantification of gastrointestinal liquid volumes and distribution following a 240 ml dose of water in the fasted state. Mol Pharm

NTP (2008). NTP Technical Report on the Toxicology and Carcinogenesis Studies of Sodium Dichromate Dihydrate in F344/N Rats and B6C3F1 Mice (Drinking Water Studies).

Parrott et al., (2009). Predicting pharmacokinetics of drugs using physiologically based modeling--application to food effects. AAPS J 11: 45-53.

Kirman et al., (2013). Physiologically based pharmacokinetic model for humans orally exposed to chromium. Chem Biol Interact 204: 13-27.

Schlosser, PM; Sasso, AF. (2014). A revised model of ex-vivo reduction of hexavalent chromium in human and rodent gastric juices. Toxicol Appl Pharmacol 280: 352-361.

Thompson et al., (2014). A chronic oral reference dose for hexavalent chromium-induced intestinal cancer. J Appl Toxicol 34: 525-536.